Ceftolozane/Tazobactam Antibiotic Activity in a Rural California Region and Improving Science Communications in Undergraduate Classrooms to Broaden Impact

UNIVERSITY OF CALIFORNIA MERCED

A dissertation submitted in partial satisfaction of the
requirements for the degree

Doctor of Philosophy in Quantitative and Systems Biology

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2021

Title: Ceftolozane/Tazobactam Antibiotic Activity in a Rural California Region and Improving Science Communications in Undergraduate Classrooms to Broaden Impact

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2021

Dedications

This work is dedicated to my husband, Mounir Alkhouri. Thank you, Mounir, for your constant love and support. If it were not for your encouragement and support, I would not have been able to get back in the grind, and not only get my Bachelor's degree, but a Doctorate degree. Thank you for all that you do, I love you.

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I. List of Abbreviations

Abb.	Description
ESBL	Extended Spectrum β-lactamase
PBP	Penicillin Binding Proteins
UTI	Urinary Tract Infection
DHMMC	Dignity Health Mercy Medical Center
DBER	Discipline Based Education Research
STEM	Science, Technology, Engineering, and Mathematics
TDM	Teacher Discourse Moves
COPUS	Classroom Observation Protocol for Undergraduate STEM
CDOP	Classroom Discourse Observation Protocol

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IV. Acknowledgments

- I would like to thank Dignity Health Mercy Medical Center in Merced, CA for providing patient isolates that made this research possible.
- I would also like to thank my advisors for their guiding and scaffolding support throughout my professional growth
    - Dr. Miriam Barlow, who was always there for me, guided me through my graduate years, on the professional and personal level. Thank you.
    - Dr. Petra Kranzfelder who gave me the opportunity to dig into DBER, funded me to do it, and guided me throughout the process. Thank you.
- Also, many thanks to my committee members:
    - Dr. Mark Sistrom who was there for me throughout my dissertation work, but especially during the crucial bioinformatics sections of my dissertation.
    - Dr. Suzanne Sindi whose continuous support and feedback on the mathematical aspects of my dissertation made my work more valuable.
    - Dr. Marcos E. García-Ojeda whose support has been continuous since my undergraduate studies. Thank you for pushing me towards greater achievements, all while keeping the door open for brainstorming ideas and casual conversations.
- Special thanks to my collaborators who helped co-author some of my dissertation chapters:
- Fabian Santiago provided outstanding and insightful mathematical expertise.
- Candace Guzman-Cole provided unpublished PCR data from her dissertation project.
- Cristine Donham provided insight and support throughout the education research.
- Their hard work and partnership throughout my dissertation work were invaluable. I hope to continue these collaborations in the future.

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VI. ABSTRACT OF THE DISSERTATION

Ceftolozane/Tazobactam Antibiotic Activity in a Rural California Region and Improving Science Education in Undergraduate Classrooms to Broaden Impact

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Doctor of Philosophy in Quantitative and Systems Biology
University of California, Merced, 2021

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Ceftolozane/tazobactam (c/t) is a fifth generation β-lactam cephalosporin and β-lactam inhibitor, recently approved in the United States for treatment of complicated Urinary Tract Infections (UTIs) and complicated intra-abdominal infections. While third generation cephalosporins are commonly used for *Enterobacteriaceae* infection treatment, fourth generation cephalosporins are used less frequently to spare them from rising antibiotic resistance, and fifth generation cephalosporins, such as c/t, are last-resort treatment options. Surveillance for c/t resistance is essential to identifying current resistance patterns and to identify causes of any observed resistance. We performed Kirby-Bauer disk diffusion tests on a collection of Extended Spectrum β-lactamases (ESBL)-producing *Enterobacteriaceae* collected mainly from urine samples at Dignity Health Mercy Medical Center (DHMMC) in Merced, CA ($n = 993$), and found low rates of c/t resistance (3.24%). We used a PCR screen to quantify the presence of four common ESBLs (bla_{TEM}, bla_{OXA}, bla_{SHV}, and $bla_{CTX\text{-}M}$) on 852 isolates and assessed their association with c/t resistance. We then screened the genomic sequences of 123 isolates and used PCR to identify the presence of four non-ESBL genes, *emrD* (gene for efflux pump), *ramR* (gene for efflux pump repressure), and *ompK35* and *ompK37* (genes for porins in bacterial outer membrane) in 96 isolates subset. We found that the presence of $bla_{CTX\text{-}M}$, bla_{SHV} independently contributed to c/t resistance. We did not find significant interactions between the non-ESBLs and c/t resistance, nor did we find any significant interactions between having a combination of ESBL and non-ESBL genes with c/t resistance.

We then wanted to find the teaching patterns in Science, Technology, Engineering, and Mathematics (STEM) classrooms. We investigated how STEM instructors, who are mainly faculty on the forefront of scientific discoveries, discuss scientific content with their students. We looked at instructional and discourse practices of 35 STEM instructors across variables such as STEM discipline, years of teaching experience, and class size. We found that chemistry instructors used more instructor-centered teaching practices (i.e., lecturing with real world examples) than biology instructors. We also found that teaching faculty use more student-centered teaching practices than lecturers. Additionally, we found that neither years of faculty teaching experience nor class size impacted teaching practices. Investigating resistance patterns to newly available antibiotics is essential for prolonging its efficacy, while studying patterns of effective communication of these scientific findings in undergraduate STEM classrooms are equally important, and both contribute to the betterment of our society.

Chapter 1: Introduction

Antibiotics history

Antibiotics have revolutionized medicine since Alexander Fleming's penicillin discovery in 1928 [1–3]. Thanks to advances in antibiotic discovery and production, common deadly illnesses, such as pneumonia and tuberculosis, could be treated effectively, and small cuts are no longer fatal if they got infected [4]. However, resistance to penicillin emerged shortly after; *in* vitro experiments in the 1940s showed the rapid emergence of penicillin resistant *Staphylococcus aureus* and *Escherichia coli* [5–12]. Resistance is the ability of infectious bacteria to grow in the presence of chemical compounds designed to kill it. The emergence and dissemination of antibiotic resistant (AR) bacteria threatens to undo these advances and cause a return to the pre-antibiotic era [6–12].

Penicillins

Penicillin, the first discovered antimicrobial chemical isolated from the fungal *Penicillium* genus, was effective against all Gram-positive and some Gram-negative pathogens [1], but with emerging resistance, new antibiotics are required. Discovery of new and effective antimicrobial compounds drove commercial production of different antibiotics, with β-lactam antibiotics prominently in the lead. β-lactams are a class of antibiotics that includes penicillins, cephalosporins, carbapenems, and monobactams, which all have a common structure of 3-carbon and 1-nitrogen ring, the β-lactam ring [13]. β-lactam antibiotics bind to penicillin-binding proteins (PBPs) in the bacterial peptidoglycan layer and interrupt the transpeptidation process, halting the crosslinking of the peptide chains in the peptidoglycan strands, causing loss of viability and cell lysis [13,14].

Cephalosporins

Cephalosporin β-lactam antibiotics are the most commonly prescribed group of antibiotics [15,16], and continue to be the preferred infection treatment option due to their high effectiveness, low cost, ease of delivery, and low toxicity with minimal side effects [16–20]. Cephalosporins have a 6-member dihydrothiazine ring attached to the β-lactam ring [20–23], with increasingly complex side chains [18,21,22] leading to their classification into generations. Lower-generation cephalosporins have more Gram-positive activity while higher-generation cephalosporins have more Gram-negative activity [18,21,22]. As resistance to the 1st generation cephalosporin was observed in the 1970's, 2nd generations cephalosporins were developed with the introduction of an α-alkoxyimino group as a side chain, increasing stability [22,24]. 3rd generation cephalosporins have even bulkier side chains and have improved activity against Gram-negative bacteria, especially when the side chains had zwitterions, having positive and negative regions [22,24,25]. Zwitterions are more common in 4th and 5th generation cephalosporins, which are more effective against resistant bacteria as they allows drug penetration into the outer membrane [22,24,25].

Antibiotic resistance

Resistance to β-lactam antibiotics could arise from decrease in influx porin activity or expression, increase in efflux pumps, altered PBPs, and/or the production of ESBLs [26,27]. ESBL enzymes hydrolyze the active β-lactam ring, rendering the antibiotic ineffective. β-lactamase evolved as early as antibiotics naturally evolved, and were discovered prior to penicillin use in clinical settings [6]. ESBLs are a major setback to β-lactam antibiotic treatment and a resistance determinant in Gram-negative bacteria [28–30]. ESBLs are the driving force for the development of all

the cephalosporin generations, in efforts to hinder the enzymes from easily reaching their target, the β-lactam ring. There are four ESBL classes, A, B, C, and D, initially classified based on molecular size and homology between active-site amino acid motifs prior to sequencing, then based on functional capabilities related to substrate and inhibitor profiles as technology evolved [28–31]. Classes A, C, and D utilize a serine in the active site, while class B requires a metal cation (Zn^{++}) [29–31]. Although there are 4 ESBL classes, their commonality is the ability to hydrolyze chemical compounds containing a β-lactam ring [28–34].

Inhibitors

β-lactamase inhibitors have been developed in addition to adding complex side chains around the β-lactam ring to aid in combating ESBLs [22,23,35,36]. These enzyme inhibitors are administered in conjunction with β-lactam antibiotics so that the inhibitor binds to the β-lactamase enzymes, allowing the antibiotic to bind to and inhibit the PBP, blocking transpeptidation and ultimately killing the bacteria [13,28,37]. β-lactamase inhibitors are structurally similar to penicillin, but have a weak antibacterial activity alone [20,28,37,38]. Inhibitors are classified as either β-lactam or non-β-lactam, and reversible or irreversible. For example, avibactam is a non-β-lactam β-lactamase inhibitor that functions by reversible acylation of the active site serine of the β-lactamase enzyme [35], while clavulanic acid, sulbactam, and tazobactam are β-lactam "suicide inactivators" of ESBLs, and function by acylation followed by rearrangement and fragmentation events [23,31]. β-lactam β-lactamase inhibitors are less effective than non- β-lactam β-lactamase inhibitors as those with a β-lactam ring are subject to hydrolysis by β-lactamase enzymes as substrate [23,28,31,35,36]. The coupling of β-lactam antibiotics with inhibitors have restored the bactericidal activity of certain antibiotics, buying time for new antibiotic development. Penicillin-inhibitor combinations such as amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam, are some β-lactam/inhibitor combinations with wide application for treatments of both community and healthcare associated infections by extended ESBL producing organisms [23]. As new cephalosporin production progresses, ceftolozane has been a promising cephalosporin for resistant microorganisms.

Ceftolozane/tazobactam

Ceftolozane/tazobactam (c/t), marketed as Zerbaxa™, is combination therapy approved in the Unites States in 2014 for treatment of complicated urinary tract infections and complicated intra-abdominal infections, and is deemed a last resort antibiotic treatment, especially for complicated urinary tract infections (UTIs) [27,39–44]. Ceftolozane is a 5th generation cephalosporin antibiotic used mainly for treatment of infections caused by Gram-negative bacteria that are resistant to conventional antibiotics including multidrug resistant (MDR) *Pseudomonas aeruginosa* and β-lactam resistant *Enterobacteriaceae* [22,45–48]. The chemical structure of ceftolozane is similar to that of ceftazidime (a 3rd generation cephalosporin), but with modified side chain in the 3rd position of the cephem rings and longer R2 side chain with increased basicity, which gives it more stability than ceftazidime and increase potency against ESBL harboring Gram-negative bacilli [45,48]. However, ceftolozane could still be compromised by certain ESBLs and carbapenemases [45,48], which is compensated for by the addition of tazobactam [22,46–48].

Urinary Tract Infections (UTIs)

UTI's are microbial infections in anatomical structures such as the bladder, ureters, and the renal pelvis [49]. In the United States, there is 6-8 million cases of UTIs yearly, and >80% of those infections are caused by *E. coli* [50,51]. Although *E. coli* is the most common etiologic infectious agent in UTIs, there are other pathogens known to cause such infections; these pathogens include *Klebsiella pneumoniae, Staphylococcus saprophyticus*, and *Proteus mirabilis* [52]. Although *K. pneumoniae* is better recognized as a cause of pneumonia, it contributes up to 5% of community-acquired UTIs, as it favors the urinary tract [52]. Antibiotic resistant UTI infectious *Enterobacteriaceae* are becoming an increasing concern as hospital-acquired UTI infections are less susceptible to empirical treatments [53] and could require complicated treatment regimens, which often results in treatment failures [50]. We obtained patient isolates from a local hospital that have been mainly isolated from urine and tested to have ESBL genes. This is a good collection to look for c/t resistance. However, we must first review c/t resistance patterns across the globe.

Surveillance Studies on c/t

To prolong the efficacy of c/t, surveillance for resistance to it should be performed promptly and periodically across the globe [54]. Since its dispersion in late 2014, many surveillance studies have been performed: a study on bloodstream isolates collected from 2011-2015 for the British Screen Advisory Council showed that 99.7% of 2676 *E. coli* and 97.6% of 1296 *Klebsiella* spp. isolates were susceptible to c/t [55]. The same study showed that almost all ESBL- and AmpC-producing *E. coli* were susceptible to c/t (97.9% and 96.6%, respectively); it also showed that some of the c/t resistant ESBL-producing *Klebsiella* possibly had larger amounts of ESBL, CTX-M variants or multiple β-lactamases leading to the observed resistance [55].

Another *in vitro* study looked at the activity of c/t against 500 *Enterobacteriaceae* (250 *E. coli*, 104 *Klebsiella* spp., 70 *Enterobacter* spp., 36 *Proteus* spp., 17 *Serratia marcescens*, 12 *Citrobacter* spp., 10 *Morganella morganii*, and 1 *Salmonella enterica)* and 500 *P. aeruginosa* collected from patients with complicated intra-abdominal, complicated urinary tract, lower respiratory tract or bloodstream infections across 10 medical centers in Spain[56]. This study showed that 94.4% of all *P. aeruginosa* were c/t susceptible, which is eight-fold more active than ceftazidime and cefepime, at least 8-fold more active than piperacillin-tazobactam, 4-fold more active than imipenem, and at least 2-fold more active than meropenem [56]. For *E. coli*, c/t demonstrated "excellent" overall activity against all 250 isolates with 99.6% susceptibility; c/t exhibited potent activity against *E. coli* with wildtype phenotype, ESBL producing phenotype, and slightly lower activity against isolates with AmpC-like phenotype compared to ESBL-producers [56]; c/t was more potent against ESBL-producing *E. coli* than were piperacillin-tazobactam, ceftazidime, cefotaxime, and cefepime [56].

A similar study that looked at c/t activity across Europe looked at 7,503 *P. aeruginosa* and 30,582 *Enterobacteriaceae* (including 11,516 *E. coli*) from 53 medical centers in 25 countries collected during 2012-18, showed generally potent activity against all isolates tested [57]. This study showed a *P. aeruginosa* susceptibility rate of 94.1% in Western Europe and 80.9% in Eastern Europe, while for *Enterobacterales,* the susceptibility rates were 94.5% in Western Europe and 79.4% in Eastern Europe. Of those they found a 99.1% susceptibility rate in Western Europe and 96.1% in Eastern Europe specifically for the *E. coli* isolates [57]. Another study done across 70 major medical centers in the United States using 1,428 *Enterobacteriaceae* (863 *K. pneumoniae* and 565

E. coli) and 2215 *P. aeruginosa* isolates showed an overall potent c/t activity against all isolates [58]. They found the susceptibility rate to be 95.9% for *P. aeruginosa*, 91.6% *K. pneumoniae* susceptibility rate and 96.4% *E. coli* [58]. The most common β-lactamase families found in their resistant *Enterobacteriaceae* isolates were CTX-M-1 family (mainly CTX-M-15), SHVs, OXA-1/OXA-30, and TEMs [58].

While most of the c/t surveillance studies showed good to excellent c/t activity specifically against *Enterobacteriaceae*, a surveillance study across 30 medical centers in China found a much lower susceptibility rate than those previously mentioned [59]. This study looked at c/t activity against 1,774 *Enterobacteriaceae* isolates with 993 *K. pneumoniae* of those 267 are carbapenem resistant (CR) isolates, and 646 *E. coli* with 28 CR isolates. They found a 72% susceptibility rate across all *Enterobacteriaceae*; of those for *K. pneumoniae* they found 52% susceptibility rates for non-CR, and 1.9% for CR isolates; and for *E. coli* they found a 90.5% susceptibility rate for non-CR isolates and 7.1% for CR isolates [59]. A summary of the surveillance studies is below:

Study	*E. coli*	*K. pneumoniae*	*Enterobacteriaceae*	*P. aeruginosa*
55	99.7 %	97.6 %	---	---
55 with ESBL and AmpC	97.9 %	96.6%	---	---
56	99.6 %	---	---	94.4 %
57 western Europe	99.1%	---	94.5%	94.1%
57 eastern Europe	96.1 %	---	79.4%	80.9%
58	96.4%	91.6%	---	95.9%
59	90.5%	52.7%	72%	88.5

Candidate Genes

Although resistance to c/t seems to be generally low, it is important to determine the causative mechanisms of resistance to maximize the useful lifespan of c/t. The most common and important mechanism by which bacteria become resistant to β-lactam antibiotics is by expressing β-lactamases, especially ESBLs, plasmid-encoded AmpC enzymes, and carbapenem-hydrolyzing β-lactamases [27,60]. Most surveillance studies have found resistance genes especially from the $bla_{\text{CTX-M}}$ β-lactamase family to be prominent in c/t resistant isolates [27,46,58,61], but also other β-lactamases such as bla_{TEMs}, bla_{SHVs} [58,61] and bla_{OXAs} [55,62] were present, though to lesser extent. Although original work on c/t showed that it is unaffected by upregulation of efflux pumps and loss of function in porin channels in *P. aeruginosa* [45,63,64], despite the documented role of efflux pumps, and loss of function in porins with β-lactam resistance in *Enterobacteriaceae* [7,17,29,34,62,65]. Although one study found a correlation between the overexpression of *AcrAB* efflux pump and c/t resistance, there is still limited data showing the effect of such non- β-lactamase resistance mechanisms on resistance to c/t [65].

The variations in c/t susceptibility rates across the globe prompt close monitoring and continuous surveillance for c/t resistance. This is especially important as there is meager development of new antibiotic [66–68], which incites procedures and techniques to preserve and prolong the efficacy of the antibiotics currently established and used. It is also vital to screen all

geographical areas for resistance to new and significant antibiotics such c/t, especially the less represented agricultural suburban communities with relation to this new antibiotic combination. Identifying the mechanisms of resistance is vital so that we can avoid contributing and fueling the selection of resistant strains, which can disseminate and become catastrophically dominant [69].

Science Communications

It is important that we continue to detect antibiotic resistance and the associated resistance mechanisms, especially on the local level; but what is also important is communicating scientific findings to the immediate community, and among our society. Discussing scientific discoveries in settings outside of academia broadens knowledge and leads to an informed public, creating savvy consumers that make informed, evidence-based decisions, especially in policy. A start to communications about scientific discoveries outside of research labs is in the undergraduate science, technology, engineering, and mathematic (STEM) classroom. Students in these classrooms are taught by instructors who are at the forefront of scientific discoveries, but are the instructors relaying exciting discoveries in their field to their students? And if they are communicating such discoveries, are they using instructional and discourse practices that are best fitted for student understanding and learning? And how do we assess and improve classroom dynamics? We can investigate by utilizing pre-existing methods in education research. . e Discipline-Based Education Research (DBER) is a body of research and researchers that are dedicated to studying classroom dynamics [70].

Discipline-Based Education Research (DBER)

DBER is a relatively new area of research that has come about in the last few decades, but bloomed drastically in recent years [71–75]. DBER investigates learning and teaching in a discipline, from a perspective that reflects the discipline's priorities, worldview, knowledge, and practices [76]. Also, DBER seeks to develop evidence-based knowledge and practices that improve teaching and learning in STEM classrooms [71]. In summary, DBER investigates the dynamics of STEM classrooms to improve teaching and learning and create an inclusive environment for the common goal of improved education for each discipline.

Instructors play a key role in facilitating classroom dynamics by conducting activities and conversations to engage their students [70]. Instructor's actions and behaviors in the classrooms could be classified as either instructor-centered or student-centered [77–80]. Instructor-centered teaching is characterized by the predominantly use of traditional methods of teaching such as formal lectures, seminars and examinations [79], where learning is viewed as a linear process, progressing steadily from "not knowing" to "knowing" [79]. In the instructor-centered teaching approach, instructors feel the need to have a rigorous course, which gives students an unmanageable amount of course content so that students resort to memorization rather than conceptualization [81]. Also in this approach, teachers provide structured material during lectures, while students take notes. Then the received "knowledge" is tested by administering examinations, where "surface" rather than "deep" level of understanding is promoted so that students only learn the minimal levels required to obtain a good grade in the course, and produce information required by the teacher [79,81,82]. This type of instructor behavior limits time for students to fully engage with course content leading to shallow learning and passive understating [77–79].

On the other hand, student-centered teaching emphasizes that concept development and deep understanding are given priority over specific skills and behaviors as the goal of instruction [79]. In addition, learners are active participants rather than passive receivers, and have the responsibility to accommodate the learning process into their own beliefs and practices [79]. In this approach, which is also known to be active learning [83], the teacher's role is to guide and assist the learning in the difficult process of constructing their individual knowledge [79]. Students experience greater learning gains and demonstrate greater engagement in class when their instructors implement student-centered teaching techniques [78,79,84]. Examples of student-centered teaching that promotes students' deeper understanding include: small group discussions, question-and-answer time with faculty, hands-on activities in the classrooms, and group projects and activities that promote student involvement [77,78,84]. Such activities engage students in dialogues that have the potential to challenge their believes and produce conceptual change, which is especially effective when multicultural issues are examined, since students are exposed to the wide variety of perspectives [81]. These activities are associated with positive consequences to learning [77,79,84,85].

The positive outcome of student-centered instruction could be due to the student's reactive role with engaging their capacities to come up with ideas, solve problems or structure various tasks [79,85]. Therefore, moving towards student-centered teaching approach requires both teacher and student modification of thinking and actions towards education; teachers need to adapt their class time to include interactions with the students and consider students' prior knowledge and background, as well as orient and guide the students' learning process [79,81]. On the other hand, students are required to participate in their own learning process to become active learners who can transfer information and knowledge to other disciplines and real-life situations [79,81]. So, the question is: can we quantify the amount of active learning instructors are enacting in their classrooms?

Classroom Observations Protocols

Structured classroom observations have been used to investigate traditional lecturing (passive) versus active learning and many observation protocols exist for this purpose. These protocols include the Practical Observation Rubric To Assess Active Learning (PORTAAL) [86] , the Decibel Analysis for Research in Teaching (DART) [87], the Classroom Observation Protocol for Undergraduate STEM (COPUS) [88], the Reformed Teaching Observation Protocol (RTOP) [89], and the Classroom Discourse Observation Protocol (CDOP) [90]. There are differences in how each of these classroom observation protocols characterizes instructional practices. Briefly, PORTAAL is intended to support STEM instructors moving from instructor-centered to active learning-based instruction [86], while DART analyzes the volume and variance of classroom sound to accurately predict the learning activities used in classrooms [87]. RTOP provides standardized means to determine the degree of student-centered and engaged learning practices using a 5-point Likert scale for 25 items [89]. COPUS on the other hand, is a non-evaluative classroom observation protocol that provides an objective account of what both instructors and students are doing during a class period. It helps capture classroom dynamics and allows for the quantification of active learning [88]. Tools like COPUS and RTOP examine the prevalence of instructional practices without assessing Teacher Discourse Moves (TDMs), whereas CDOP specifically measures TDMs [90]. TDMs are specific conversational strategies used by instructors to foster an engaging active learning classroom and mechanisms for promoting student thinking and generation of knowledge [90,91];

CDOP is a relatively new tool that quantifies TDMs and the content-related discussions in the undergraduate STEM classroom [90].

COPUS has been popular among DBER researchers as it provides concrete quantification to what is physically happening in the classroom; it quantifies the time instructors and students engage in various activities within STEM courses [88,92]. COPUS categorization was collapsed into four categories that describe instructors' behavior's: *presenting, guiding, administering* and *other* [93]. *Presenting* is considered instructor-centered behavior, while *guiding* is a student-centered behavior that actively engage students [93]. *Administering* and *other* are not pertaining to instructional behaviors, but rather for classroom logistics. Additionally, CDOP is the only tool currently available to quantify TDMs. CDOP categorizes instructor discourse into three discourse approaches: 1) *authoritative, non-interactive*; 2) *authoritative, interactive;* and 3) *dialogic, interactive*. *Authoritative* approaches are considered teacher-centered, while the *dialogic, interactive* approach is student-centered [90]. Equipped with these classroom observation protocols, individual faculty, departments, or schools and colleges in higher education can study patterns of instructional and discourse practices to have targeted professional development, improve teaching, and pushing towards more student-centered teaching practices.

Studies Using COPUS

COPUS has been used to study instructional patterns within and across STEM disciplines, and across variables such as instructors' years of teaching experience, their appointment track, and class size [78,94–96]. Prominent in their findings was that chemistry instructors used more instructor-centered teaching practices, while biology was geared more toward student-centered teaching [94,95]. A study using COPUS and CDOP found differences within biology instructors; they found that biology instructors *guide* their students but with an *authoritative* discourse approach [97]. Also, while it has been found that having a small or large class size does not impact instructional practices [94–96], instructors' appointment line has an effect [98]; Xu and Solanki showed that teaching faculty implement more active learning practices in their STEM classrooms than research faculty and lecturers. Moreover, Moreover, it has been shown that the amount of active learning in a classroom also increases with increasing teaching experience [99,100].

It is important to investigate course and instructors' characteristics across STEM classrooms in many settings to understand the teaching patterns and classroom dynamics, for targeted support and guidance towards student-centered teaching approaches. The studies mentioned above have mainly looked at patterns in instructional practices across STEM disciplines, class size, years of instructor's teaching experience, and appointment line [94,96,98,100], but only Kranzfelder et al., looked at the instructional discourse along with instructional practices [97]. The limitations to this last study is that it only included biology instructors mainly teaching introductory biology courses at a research-intensive, predominantly white institution [97]. What would the patterns of both the *instructional and discourse* practices look like across the variables mentioned (STEM discipline, Class size, years of faculty teaching experience, and appointment line or instructor type) at a Hispanic Serving Institution? It is important to investigate these patterns locally to understand current instructional trends and allow for targeted professional development for the common goal of improved student performance outcomes with increased content retention to raise informed generations.

Chapter 2: Molecular Surveillance and Associations of ESBL Genes with Ceftolozane/Tazobactam Resistance

Abstract

Ceftolozane/tazobactam (c/t) is a new and potent antimicrobial combination therapy of a fifth generation β-lactam cephalosporin and an inhibitor approved in 2014 for treatment of multidrug resistant (MDR) *Enterobacteriaceae*, especially resistant intra-abdominal and urinary tract infections. The aim of this study is to assess c/t activity for *Enterobacteriaceae* isolates collected mainly from urinary tract infections in an agricultural-heavy region in California (USA) between 2013-2020, and to examine the association of c/t resistance with four common β-lactamase resistance genes. We tested 993 ESBL producing *Enterobacteriaceae* isolates (885 *E. coli*, 94 *K. pneumoniae*, 14 other) for c/t susceptibility by Kirby-Bauer disk diffusion and PCR screened of four common β-lactamase resistance genes (bla_{TEM}, bla_{OXA}, bla_{SHV}, and $bla_{\text{CTX-M}}$) for 852 of the isolates. We found that most isolates were susceptible to c/t (58.53%), while 38.24% had intermediate resistance, and 3.24% were resistant. Analysis of genotypic data show bla_{SHV} and $bla_{\text{CTX-M}}$ are independently associated with the observed c/t resistance. We also found common co-occurrence of c/t resistance with other antibiotics such as piperacillin/tazobactam, ertapenem, imipenem, and amikacin. Although c/t demonstrated strong antimicrobial activity against *Enterobacteriaceae*, the high percentage of isolates with intermediate susceptibility warrants close monitoring and additional surveillance for c/t resistance.

Introduction

When new antibiotics are introduced for clinical consumption, resistance to them arises very rapidly, usually within 3 years [101–103]. Despite strong clinical need, few new antibiotics are introduced into clinics [66–68]. Ceftolozane/tazobactam (c/t) is a new antibiotic/inhibitor combination that received FDA approval in December, 2014 for treatment of complicated Intra-Abdominal Infections (cIAI) and complicated Urinary Tract Infections (cUTI) [27,32,45,58]. This combination is particularly useful for treating Extended Spectrum Beta Lactamase (ESBL) producing *Enterobacteriaceae* and MDR *Pseudomonas aeruginosa* but not carbapenem-producing *Enterobacteriaceae* (CREs) [32,39,45,58,62]. Ceftolozane is a 5$^{\text{th}}$ generation cephalosporin β-lactam that targets penicillin binding proteins, while tazobactam is an inhibitor that protects ceftolozane from hydrolysis by blocking β-lactamase enzymes [32,45,58]. When a new antibiotic such as c/t is approved for clinical treatment, it is important to survey resistance to it, both to understand what susceptibility testing panels should look like and to understand how to maintain efficacy of treatment [54].

Although concurrent resistance to cephalosporins and inhibitors is low due to the synergistic biochemical effects of drug combination therapies [33,104–106] and limited usage of c/t as a last resort antibiotic treatment [40], resistance to it has been observed. Surveillance of 500 *Enterobacteriaceae* and 500 *P. aeruginosa* from patients in Spain, showed 94.4% of *P. aeruginosa* and 99.6% of *Enterobacteriaceae* were susceptible to c/t [56]. Another c/t surveillance of 30,582 *Enterobacteriaceae* across Europe showed 94.5% and 79.4% rates of susceptibility in Western and Eastern Europe, respectively [57]. In the US, surveillance of 1,428 *Enterobacteriaceae* isolates collected across 70 United States medical centers found that 91.9% *K. pneumoniae* and 96.4% *E.*

coli were susceptible to c/t [58]. However, not all regions in the world show low resistance; a study of 1,774 *Enterobacteriaceae* isolates from 30 medical centers in China found a much higher rate of resistance to c/t: 8.6% *E. coli* and 43.7% *K. pneumoniae* [59]. These varied rates show geographic disparities in resistance to this treatment option.

Studies that examined the molecular mechanisms of resistance to c/t showed that it was effective against Gram-negative bacilli harboring β-lactamases such as TEM-1 and SHV-1, and those showing overexpression of AmpC [45]. Early experiments on ceftolozane's activity against *E. coli* strains bearing narrow spectrum β-lactamase and ESBLs such as TEM1-9, SHV1-4, OXA-1, -2, and CTX-M 3, -18, showed reduced antimicrobial activity when TEM3-9; SHV2-4; OXA-2; CTX-M-3, -18 were present, while the activity of imipenem was not affected by the presence of any ESBLs [45]. The activity of ceftolozane against Gram-negative bacteria is either retained or enhanced upon the addition of the inhibitor tazobactam [40,45,107]. In another study, the addition of tazobactam to ceftolozane showed reduced minimum inhibitory concentrations (MICs) against *Enterobacteriaceae* harboring CTX-M, SHV, TEM, and PER-1 [45,46]. Moreover, another study showed that only 66.7% of 91 isolates carrying bla_{SHV} were inhibited by c/t [108]. Taken together, these data suggest that CTX-Ms and SHV's are major contributors to c/t resistance.

The goal of this study is to survey the frequency of c/t resistance in a repository of ESBL+ isolates collected from Dignity Health Mercy Medical Center (DHMMC), a hospital serving an agricultural community in the San Joaquin Valley of California. We combine the surveillance data with susceptibility testing for other antibiotics and molecular surveillance of important β-lactamases (bla_{TEM}, bla_{OXA}, bla_{SHV}, and $bla_{\text{CTX-M}}$) to determine the relationships between resistance phenotypes and carriage of common β-lactamases.

Results

We measured the zone of inhibition (ZI) of 993 ESBL+ isolates tested with the Kirby-Baur Disk Diffusion susceptibility test and found that 3.24% (n = 33) of the isolates are c/t resistant (ZI ≤ 17mm), 38.24% (n = 578) are with intermediate c/t resistance (ZI = 18-20mm), and 58.53% (n = 382) are c/t susceptible (ZI ≥ 21mm) according to Clinical and Laboratory Standards Institutute (CLSI) breakpoints. The distribution of *Enterobacteriaceae* c/t resistance in our collection was 1.86% *E. coli* isolates and 0.98% *K. pneumoniae* isolates.

We determined the empirical frequencies of resistance to other important antibiotics from the minimum inhibitory concentrations (MICs)[110] provided by DHMMC (Table 11). Critically, we observed a much lower rate of resistance to the penicillin/inhibitor combination piperacillin/tazobactam (pip/tazo) (0.0551) that was only slightly higher than c/t (0.0324). However, we did observe a higher rate of resistance for the ampicillin/sulbactam (amp/sul) (0.5902) combination.

We used the complete set of resistance profiles to compute conditional frequencies (Table 11) of c/t resistance when other resistance phenotypes are observed. We found that carbapenem resistant isolates have a higher rate of resistance to c/t (imipenem 0.5 and ertapenem 0.44), suggesting that CRE's are the most likely isolates to exhibit c/t resistance. Other research also shows c/t resistance when resistance to ceftazidime and imipenem is observed [111]. Overall, we found a high rate of co-resistance in our collection between c/t, pip/tazo, ertapenem, imipenem, and amikacin. This result indicates the need for c/t susceptibility tests prior to treatment with c/t.

We also computed the conditional frequency of resistance to other antibiotic treatments when resistance to c/t is observed (Table 11). Despite the increased risk of resistance associated with carbapenems and penicillin/inhibitor combination, these antibiotics remain the treatment options with the greatest probability of being effective when c/t resistance is observed. This is supported by research showing that c/t is a viable option to spare the use of carbapenems [112] and vice versa.

Conclusively, c/t is an effective drug against *Enterobacteriaceae* isolates that are resistant to other antibiotics, including carbapenem resistant ones. Additionally, if carbapenem resistance is observed, c/t may be a good treatment choice.

Table 11. Empirical Frequency of Resistance. Column 2: Empirical frequency of resistance to seventeen β-lactam antibiotics. Column 3: conditional empirical frequency of c/t resistance given resistance to each of the other sixteen antibiotics. Column 4: conditional empirical frequency of resistance to each of the other sixteen antibiotics given resistance to c/t.

Antibiotic	**Frequency of Resistance**	**Frequency of c/t resistance given resistance to each antibiotic**	**Frequency of resistance to each antibiotic given c/t resistance**
Ceftolozane/Tazobactam	0.0324	1.0000	1.0000
Ampicillin	0.9858	0.0271	0.8824
Ampicillin/Sulbactam	0.5902	0.0361	0.7059
piperacillin/Tazobactam	0.0551	0.1452	0.2647
Cefazolin	0.9698	0.0275	0.8824
Ceftazidime	0.9431	0.0273	0.8529
Ceftriaxone	0.9591	0.0278	0.8824
Cefepime	0.9431	0.0273	0.8529
Ertapenem	0.0080	0.4444	0.1176
Imipenem	0.0053	0.5000	0.0882
Amikacin	0.0080	0.1111	0.0294
Gentamicin	0.3476	0.0281	0.3235
Tobramycin	0.3173	0.0336	0.3529
Ciprofloxacin	0.8213	0.0260	0.7059
Levofloxacin	0.8151	0.0251	0.6765
Nitrofurantoin	0.0800	0.0778	0.2059
Trimethoprim/Sulfamethoxazole	0.6400	0.0264	0.5588

We sought to obtain greater understanding of the genetic basis for the c/t resistance phenotype by analyzing the relationship between c/t and ZI as a function of the resistance genes bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{OXA}. The distribution of these resistance genes in the tested collection is represented in Figure 1. We began by looking at the individual effects of each resistance gene on the ZI measurements (Figure 10, Table 12). From Figure 10 it appears that the presence of the genes bla_{SHV} and $bla_{CTX\text{-}M}$ both lead to lower ZI measurements indicating resistance, while no difference is observed when looking at the presence/absence of the genes bla_{TEM} and bla_{OXA}. We observed an average drop of about 1 mm in ZI for isolates that contained the bla_{SHV} gene and an average drop of about 0.60 mm in the ZI associated with the $bla_{CTX\text{-}M}$ gene when compared against isolates that did not have each bla_{SHV} or $bla_{CTX\text{-}M}$ respectively (Table 12, $p < 0.05$).

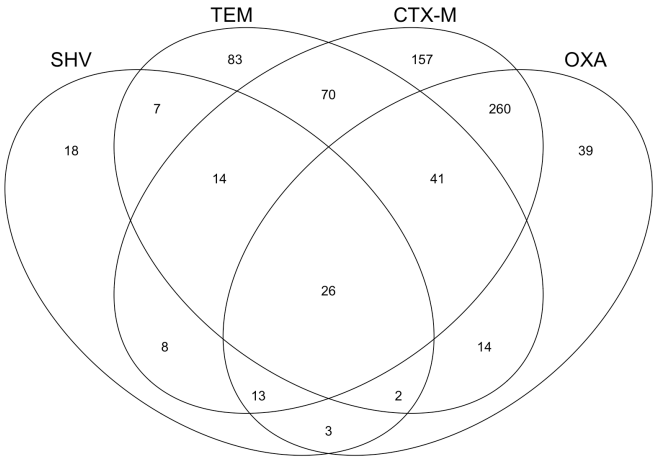


Figure 9. Combinations of Resistance Genes in 852 Clinical Isolate from the DHMMC

Figure 10. Distribution of Zone of Inhibition (mm) Measurements by Resistance Gene Presence/Absence. Resistance gene presence is indicated by [+] and resistance gene absence is indicated by [−]. The diamond (⋄) indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The Clinical Laboratory Standards Institute regions for resistance classification are labeled: susceptible ([**S**]), intermediate ([**I**]), and Resistant ([**R**]).

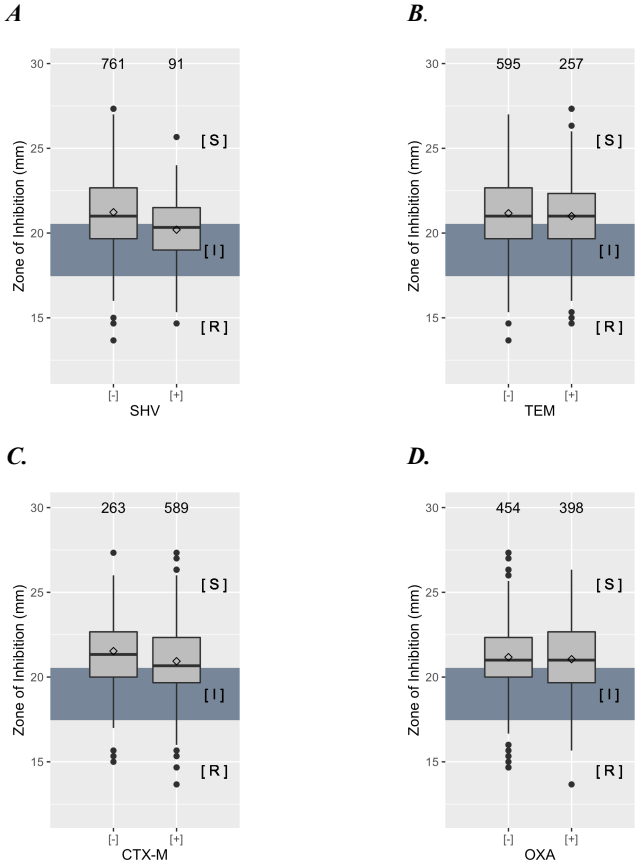


Table 12. Test for Mean Differences Between Zone of Inhibition (mm) Measurements by Presence[+]/Absence[−] of Individual Resistance Gene. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value. The asterisk (*) indicates a *p*-value <0.05.

Gene	**μ([−]) − μ([+])**	**p-value**	**95% CI**
bla$_{SHV}$	1.031	7.344e-06*	(0.580,1.481)
bla$_{TEM}$	0.169	2.812e-01	(-0.138,0.475)
bla$_{CTX-M}$	0.596	1.103e-04*	(0.294,0.898)
bla$_{OXA}$	0.121	4.016e-01	(-0.161,0.403)

While looking at individual genes gives insight into the possible effects of each resistance gene on the ZI measurements in the presence of c/t, there is a possibility for epistatic interactions between resistance genes. Therefore, we also quantified the combined effects of these resistance genes. We studied the combined effects of two gene presence ([+]) and absence ([−]) combinations, leading to the six possible combination of (1) bla_{TEM} and bla_{OXA} (bla_{TEM}: bla_{OXA}), (2) bla_{SHV} and bla_{TEM} (bla_{SHV}: bla_{TEM}), (3) bla_{SHV} and bla_{OXA} (bla_{SHV}: bla_{OXA}), (4) bla_{TEM} and bla_{CTX-M} (bla_{TEM}: bla_{CTX-M}), (5) bla_{CTX-M} and bla_{OXA} (bla_{CTX-M}: bla_{OXA}), and (6) bla_{SHV} and bla_{CTX-M} (bla_{SHV}: bla_{CTX-M}). The figures and tables for the analysis of three and four gene combinations are included in the supplemental materials (figures S1-5, tables S1-5) From these analyses, we see that the individual presence of bla_{TEM}, and bla_{OXA}, do not have a detectable effect on the ZI measurements (Figure 11, and Table 13).

Figure 11. (bla_{TEM}: bla_{OXA}): Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by [+] and resistance gene absence is indicated by [−]. The diamond (⋄) indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible ([**S**]), intermediate ([**I**]), and Resistant ([**R**]).

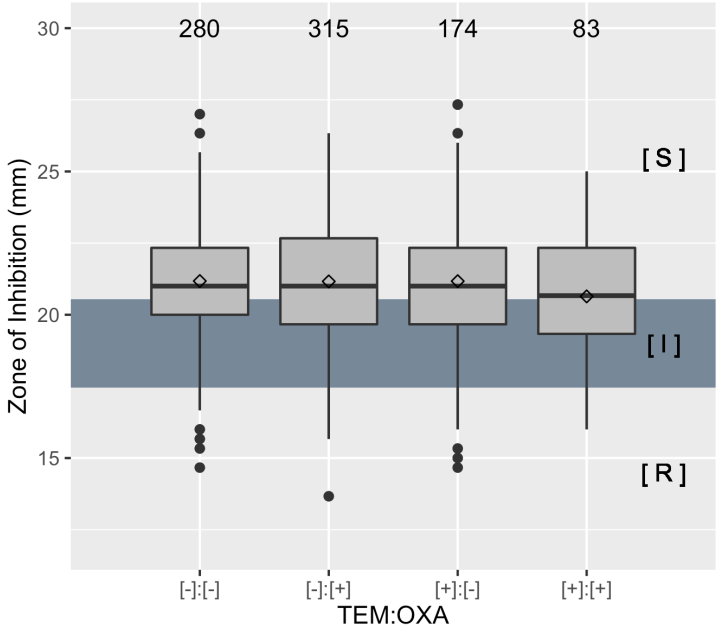


Table 13. (bla_{TEM}: bla_{OXA}): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by bla_{TEM} and bla_{OXA} Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value.

bla_{TEM}: bla_{OXA}	Difference	*p*-value	95% CI
$\mu([-]:[-]) - \mu([-]:[+])$	0.013	0.9998	(-0.428,0.455)
$\mu([-]:[-]) - \mu([+]:[-])$	0.004	1.0000	(-0.515,0.523)
$\mu([-]:[-]) - \mu([+]:[+])$	0.536	0.1708	(-0.136,1.208)
$\mu([-]:[+]) - \mu([+]:[-])$	-0.009	1.0000	(-0.517,0.499)
$\mu([-]:[+]) - \mu([+]:[+])$	0.522	0.1796	(-0.141,1.186)
$\mu([+]:[-]) - \mu([+]:[+])$	0.532	0.2.260	(-0.186,1.249)

When looking at the combined presence/absence of bla_{SHV} and either bla_{TEM} or bla_{OXA} (Figure 12, and Figure 13), we find that it is the presence of bla_{SHV} that leads to decreases in ZI measurements. In the bla_{SHV} and bla_{TEM} combinations (Figure 12), and the bla_{SHV} and bla_{OXA} combinations (Figure 13), we see that the presence of bla_{SHV} drives the decrease in average ZI measurements (Table 14 and Table 15, respectively, $p < 0.05$). looking at Table 14 with isolates having bla_{SHV} that lack bla_{TEM} (condition $[+]:[-]$), and then have both genes (condition $[+]:[+]$), we can see that presence of bla_{TEM} has no significant effect on average difference in ZI measurements, as the significance comes from having bla_{SHV}. Similarly, in looking at the combined effects of bla_{SHV} and bla_{OXA} on ZI measurements, it is the presence of bla_{SHV} that drives a decrease in the ZI measurements (Table 15).

Figure 12. (bla_{SHV}: bla_{TEM}): Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by [+] and resistance gene absence is indicated by [−]. The diamond (⋄) indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible ([**S**]), intermediate ([**I**]), and Resistant ([**R**]).

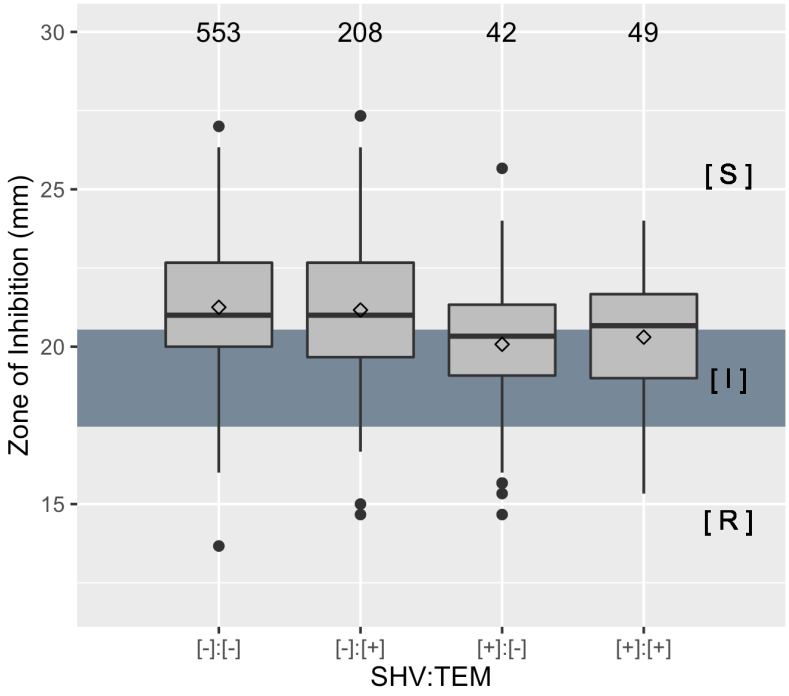


Table 14. (bla_{SHV}: bla_{TEM}): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by bla_{SHV} and bla_{TEM} Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value.

***bla*$_{SHV}$: *bla*$_{TEM}$**	**Difference**	***p*-value**	**95% CI**
$\mu([-]:[-]) - \mu([-]:[+])$	0.087	0.9551	(-0.346,0.521)
$\mu([-]:[-]) - \mu([+]:[-])$	1.177	0.0022	(0.324,2.030)
$\mu([-]:[-]) - \mu([+]:[+])$	0.95	0.0115	(0.155,1.744)
$\mu([-]:[+]) - \mu([+]:[-])$	1.09	0.0103	(0.188,1.991)
$\mu([-]:[+]) - \mu([+]:[+])$	0.862	0.0438	(0.016,1.709)
$\mu([+]:[-]) - \mu([+]:[+])$	-0.227	0.9540	(-1.348,0.893)

Figure 13. (bla_{SHV}: bla_{OXA}): Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by [+] and resistance gene absence is indicated by [−]. The diamond (⋄) indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible ([**S**]), intermediate ([**I**]), and Resistant ([**R**]).

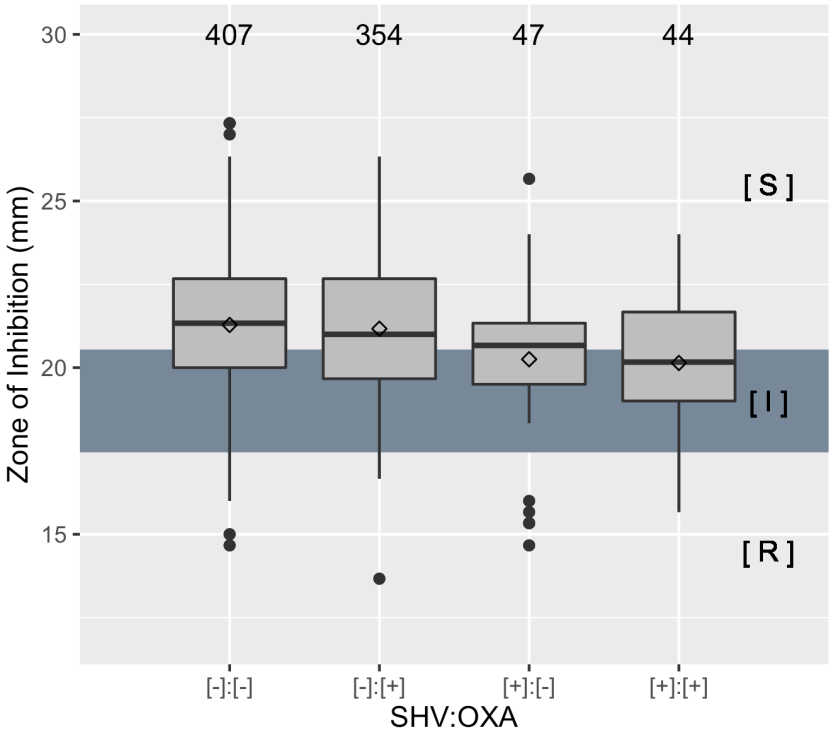


Table 15. (bla_{SHV}: bla_{OXA}): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by bla_{SHV} and bla_{OXA} Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value. The asterisk (*) indicates a *p*-value <0.05.

bla_{SHV}: bla_{OXA}	Difference	*p*-value	95% CI
$\mu([-]:[-]) - \mu([-]:[+])$	0.114	0.87480	(-0.274,0.501)
$\mu([-]:[-]) - \mu([+]:[-])$	1.03	0.00697	(0.209,1.850)
$\mu([-]:[-]) - \mu([+]:[+])$	1.141	0.00296	(0.296,1.987)
$\mu([-]:[+]) - \mu([+]:[-])$	0.916	0.02308	(0.089,1.743)
$\mu([-]:[+]) - \mu([+]:[+])$	1.027	0.01047	(0.176,1.879)
$\mu([+]:[-]) - \mu([+]:[+])$	0.112	0.99410	(-1.006,1.229)

With respect to individual resistance genes, we found that the *bla*$_{TEM}$ gene was not associated with a decrease in ZI measurements; however, when we considered the combined effects of *bla*$_{TEM}$ and *bla*$_{CTX-M}$, we observed that the greatest effect on the average ZI measurements occurred when both the *bla*$_{TEM}$ and *bla*$_{CTX-M}$ genes were present (condition [+]: [+], Table 16, p <0.05, Figure 6). While having both *bla*$_{TEM}$ and *bla*$_{CTX-M}$ genes is associated with a lower ZI measurement, suggesting that that *bla*$_{CTX-M}$ is the driving factor for resistance. This can be seen from the lack of significant difference in the comparison of ZI measurements of isolates without the *bla*$_{TEM}$ gene but harbor the *bla*$_{CTX-M}$ gene (condition [−]: [+]) against isolates that contain both genes (condition [+]: [+]). However, when comparing the ZI measurements when both *bla*$_{TEM}$ and *bla*$_{CTX-M}$ genes are present (condition [+]: [+]) with the absence of *bla*$_{CTX-M}$ (condition [+]: [−]), we observe a statistically significant drop in the average ZI measurement difference (Table 16, p <0.05). These observations remained consistent when we factor out the effects of the *bla*$_{SHV}$ gene (see Supplementary Table 1, p <0.05). This result may indicate an epistatic interaction between the *bla*$_{TEM}$ and *bla*$_{CTX-M}$ genes.

Figure 14. (*bla*$_{TEM}$: *bla*$_{CTX-M}$): Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by [+] and resistance gene absence is indicated by [−]. The diamond (⋄) indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible ([**S**]), intermediate ([**I**]), and Resistant ([**R**]).

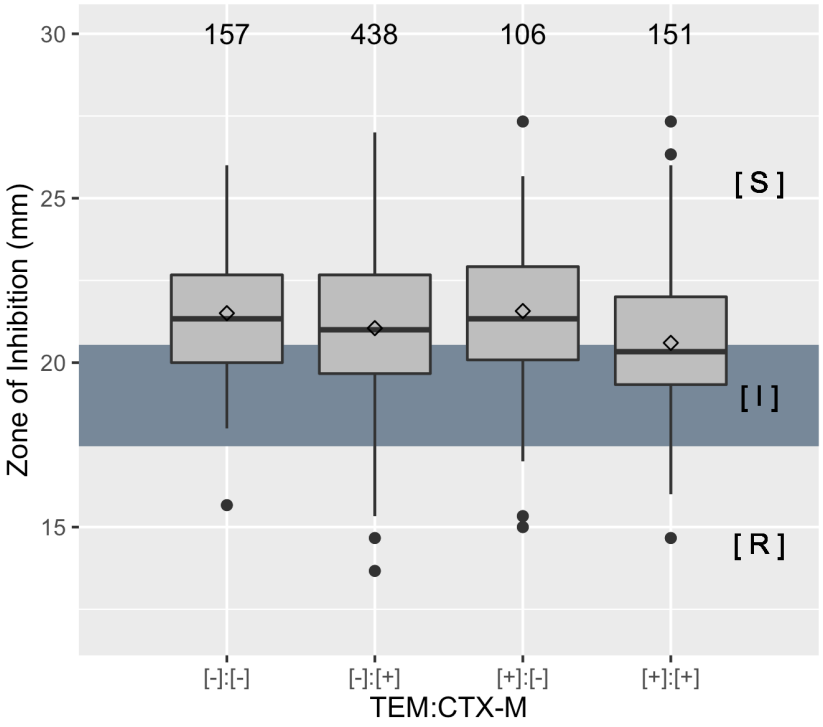


Table 16. (bla_{TEM}: $bla_{CTX\text{-}M}$): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by bla_{TEM} and $bla_{CTX\text{-}M}$ Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value. The asterisk (*) indicates a *p*-value <0.05.

bla_{TEM}: $bla_{CTX\text{-}M}$	Difference	*p*-value	95% CI
$\mu([-]:[-]) - \mu([-]:[+])$	0.455	0.08523	(-0.041,0.951)
$\mu([-]:[-]) - \mu([+]:[-])$	-0.065	0.99460	(-0.735,0.605)
$\mu([-]:[-]) - \mu([+]:[+])$	0.903	0.00078	(0.295,1.510)
$\mu([-]:[+]) - \mu([+]:[-])$	-0.52	0.09436	(-1.097,0.057)
$\mu([-]:[+]) - \mu([+]:[+])$	0.448	0.10130	(-0.055,0.950)
$\mu([+]:[-]) - \mu([+]:[+])$	0.968	0.00133	(0.292,1.643)

Looking at Table 19, we see that there is a positive relationship between $bla_{CTX\text{-}M}$ and bla_{OXA} resistance genes (p<0.05). However, when looking at the effects of their presence in combination on the ZI measurements (Figure 15, Table 17), we can conclude that it is the presence of $bla_{CTX\text{-}M}$ that leads to lower average zone of inhibition measurements (Table 17, p<0.05). This can be seen in the lack of significance in the comparison of isolates that do not have the $bla_{CTX\text{-}M}$ or the bla_{OXA} gene (condition $[-]:[-]$) with isolates that have the bla_{OXA} gene (condition $[-]:[+]$).

Figure 15. (*bla*$_{\text{CTX-M}}$: *bla*$_{\text{OXA}}$): Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by [+] and resistance gene absence is indicated by [−]. The diamond (⋄) indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible ([**S**]), intermediate ([**I**]), and Resistant ([**R**]).

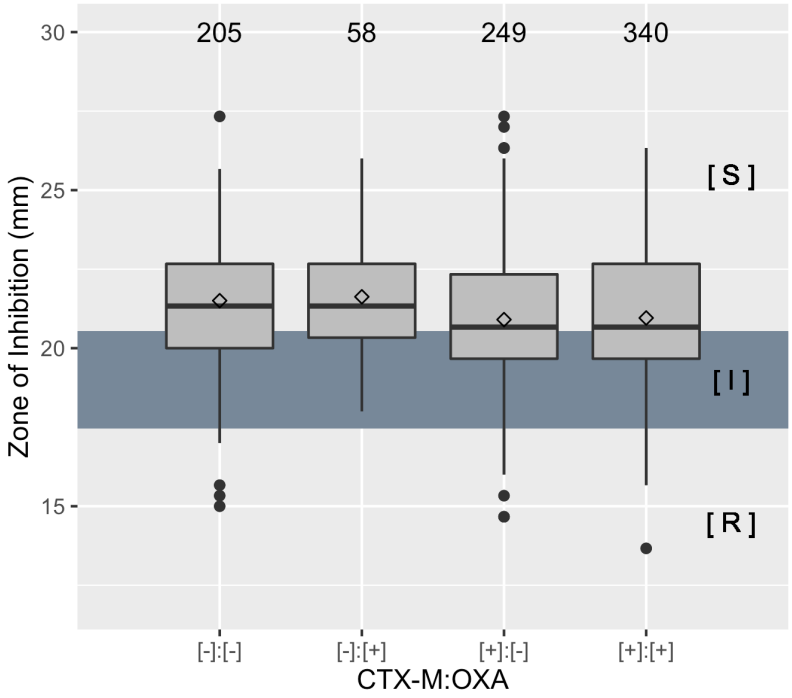


Table 17. (CTX- *bla*$_{\text{CTX-M}}$): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{OXA}}$ Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value.

***bla*$_{\text{CTX-M}}$: *bla*$_{\text{OXA}}$**	**Difference**	***p*-value**	**95% CI**
$\mu([-]:[-]) - \mu([-]:[+])$	-0.123	0.97860	(-0.918,0.672)
$\mu([-]:[-]) - \mu([+]:[-])$	0.599	0.01217	(0.095,1.103)
$\mu([-]:[-]) - \mu([+]:[+])$	0.547	0.01565	(0.074,1.020)
$\mu([-]:[+]) - \mu([+]:[-])$	0.722	0.08084	(-0.057,1.502)
$\mu([-]:[+]) - \mu([+]:[+])$	0.67	0.10590	(-0.089,1.429)
$\mu([+]:[-]) - \mu([+]:[+])$	-0.052	0.99060	(-0.498,0.394)

In the analysis above we observed that the presence *bla*$_{\text{SHV}}$, *bla*$_{\text{CTX-M}}$, and the simultaneous presence of both *bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX-M}}$ genes are associated with a decrease in average ZI

measurement. From Figure 16 and Table 18, we see that the presence of either *bla*$_{SHV}$, or *bla*$_{CTX-M}$ leads to a decrease in average ZI over not having either gene. However, the presence of the *bla*$_{SHV}$ gene leads to a larger average drop in ZI than in isolates with the *bla*$_{CTX-M}$ gene (Table 18, $p < 0.05$). This effective benefit to resistance can be observed in the comparison of isolates that have both resistance genes (condition [+]: [+]) and isolates that have one of these two genes (condition [+]: [−] or [−]: [+]). Having both genes versus only *bla*$_{CTX-M}$ leads to greater resistance, lower ZI measurements ($p < 0.05$). However, isolates that contain *bla*$_{SHV}$ and no *bla*$_{CTX-M}$, when compared against isolates that contain both genes, leads to nonsignificant difference in average ZI measurements, which may indicate an additive interaction between these genes.

Figure 16. (*bla*$_{SHV}$: *bla*$_{CTX-M}$): Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by [+] and resistance gene absence is indicated by [−]. The diamond (⋄) indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible ([**S**]), intermediate ([**I**]), and Resistant ([**R**]).

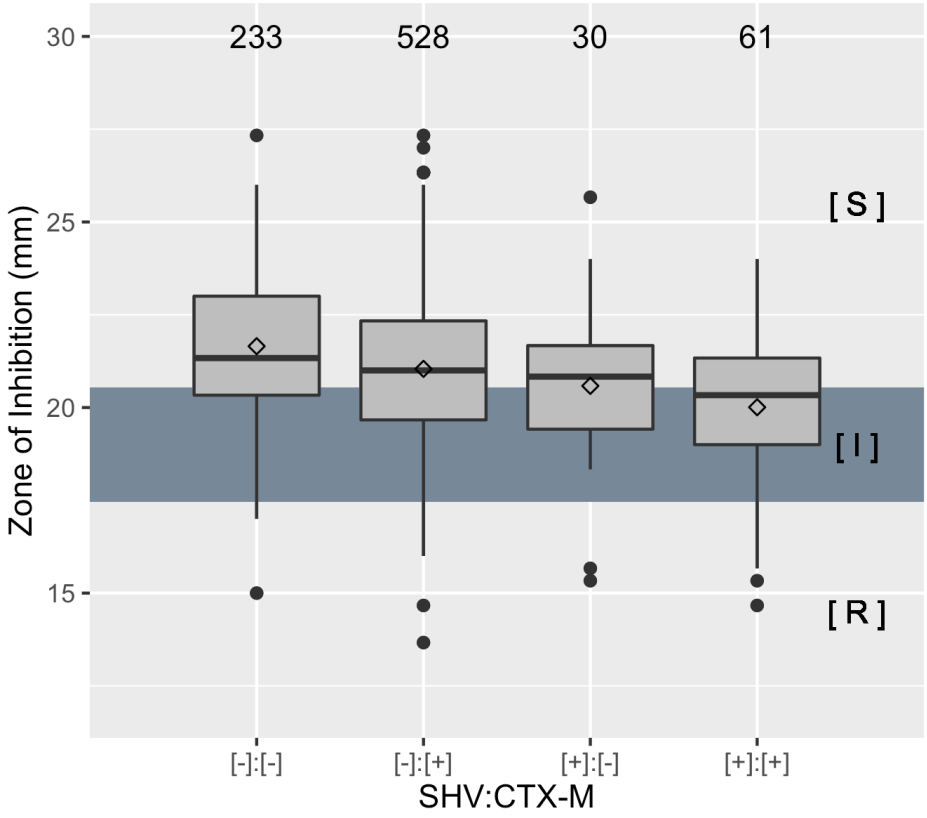


Table 18. (bla_{SHV}: bla_{CTX-M}): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by bla_{SHV} and bla_{CTX-M} Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value. The asterisk (*) indicates a *p*-value <0.05.

bla_{SHV}: bla_{CTX-M}	Difference	*p*-value	95% CI
$\mu([-]:[-]) - \mu([-]:[+])$	0.611	0.00091	(0.196,1.026)
$\mu([-]:[-]) - \mu([+]:[-])$	1.069	0.03689	(0.045,2.093)
$\mu([-]:[-]) - \mu([+]:[+])$	1.644	0.00000	(0.885,2.404)
$\mu([-]:[+]) - \mu([+]:[-])$	0.458	0.63500	(-0.533,1.449)
$\mu([-]:[+]) - \mu([+]:[+])$	1.033	0.00116	(0.319,1.747)
$\mu([+]:[-]) - \mu([+]:[+])$	0.575	0.59200	(-0.603,1.753)

Table 19. Phi Coefficient Summary for 852 Clinical Isolates. The phi coefficient, the associated p-value, and a 95% confidence interval is presented for each resistance gene pair comparison. p-value <0.05 indicates significance

Genes	Phi Coefficient	*p*-value	CI
bla_{SHV}:bla_{TEM}	0.178	1.584×10^{-07}	(0.113&0.243)
bla_{SHV}:bla_{CTX-M}	-0.016	0.6470	(-0.083&0.052)
bla_{SHV}:bla_{OXA}	0.011	0.7407	(-0.056&0.078)
bla_{TEM}:bla_{CTX-M}	-0.148	1.51×10^{-05}	(-0.213&-0.081)
bla_{TEM}:bla_{OXA}	-0.190	2.317×10^{-08}	(-0.254&-0.124)
bla_{CTX-M}:bla_{OXA}	0.330	3.924×10^{-23}	(0.269&0.389)

Finally, do we see an increase in resistance after c/t FDA approval in 2014? Despite having a relatively low frequency of c/t resistant isolates (3.24%), it was of interest to us to look at the frequency of c/t resistance over the years. In 2013, prior to approval, we see resistance in 9 (8.25%) of isolates collected that year. If we consider the frequency of resistant isolates prior to FDA approval as our baseline, any significant increase in frequency indicates developing c/t resistance. We do not see any significant increase in the frequency of c/t resistant isolates suggesting c/t maintains its efficacy. Compared to our baseline, isolates from 2014 to 2018 show a significant decrease in c/t resistance, while isolates from 2019 show no significant difference. These data suggest the continued potent activity of c/t as antimicrobial therapy; having more resistant isolated in the year prior to c/t approval than in subsequent years suggests that c/t resistance does not seem to be driven by exposure.

Discussion

Our results agree with the frequencies of c/t resistance observed in similar studies across the United States and Europe [56,58]. The rate of resistance across all *Enterobacteriaceae* clinical isolates is likely lower than 3.24% because our collection emphasizes ESBL+ *Enterobacteriaceae* that are likely predisposed for c/t resistance. This may also explain the relatively high percentage (38.24%) with intermediate resistance to c/t. We found that resistance in these clinical isolates has been consistently low over the past 6 years giving us reason to believe that this treatment will continue to be effective in the immediate future, as resistance did not seem to be increasing after c/t deployment for clinical use.

The conditional frequencies of c/t resistance and 16 other antibiotics showed that even when the frequency of resistance to many other antibiotics is high, while the frequency of resistance to c/t continues to remain low. The combination of antibiotic/inhibitor treatments showed lower resistance than those without an inhibitor for the β-lactam antibiotics, and carbapenem resistance was low overall. The fact that c/t resistance was low is likely due to low c/t usage in the area served by DHMMC. Isolates that are carbapenem resistant show increased c/t resistance frequency, indicating that co-resistance between c/t and carbapenems is likely. These findings are also supported by other studies showing that carbapenem resistant *Enterobacteriaceae* are resistant to c/t [32,62,113]. It is worth noting that the frequency of carbapenem resistance when c/t resistance is observed is lower than it is for other antibiotics, which may explain the observed low rate of c/t resistance in our collection. This indicates that although carbapenem and c/t resistance may co-exist, carbapenems may be the best treatment option when c/t resistance is observed, and vice versa [112]. It is possible that other generalized resistance determinants, such as the presence of porins and efflux pumps, could play a role in the co-occurrence of resistance to c/t and carbapenems [65].

While investigating the molecular cause of the observed resistance, we found that isolates that contained either the bla_{SHV} or $bla_{\text{CTX-M}}$ genes alone (Table 2?), or the co-presence of bla_{TEM} and $bla_{\text{CTX-M}}$ genes, are associated with a smaller c/t ZI, indicating a relationship between carriage of these genes and c/t resistance, similar to previous studies [40,42,108]. Although $bla_{\text{CTX-Ms}}$ and bla_{SHVs} are important for c/t resistance, their contribution is not sufficient for clinical resistance as shown by the many $bla_{\text{CTX-M}}$ and bla_{SHV}-positive isolates in our collection that remain susceptible to c/t (Figure 1).

While there is a low frequency of c/t resistance in our collection, it is likely that if the usage of c/t increases, there will be a corresponding increase in the frequency of isolates with $bla_{\text{CTX-M}}$ or bla_{SHV} genes and the co-occurrence of bla_{TEM} and $bla_{\text{CTX-M}}$ genes in clinical populations of bacteria. Thus, it is vital to have continuous surveillance of all new antibiotics to prolong the efficacy of these antibiotics.

Methods and Materials

We assessed the resistance profiles to c/t from a repository of 1,250 *Enterobacteriaceae* ESBL+ patient isolates collected from 2013-2020 at DHMMC, a hospital in Merced, California (USA), that serves this city and the surrounding agricultural communities. Because our isolates are enriched for ESBL+ isolates, it is an opportune collection to look for c/t resistance. In our collection, ~90% of the samples are *E. coli*, ~5% *K. pneumoniae* and ~5% are other species. All isolates have been tested for antibiotic susceptibility on the Vitek2 [110] at DHMMC for 16 common antibiotics (4 cephalosporins, 3 penicillin's, 2 carbapenems, 3 aminoglycosides, 2 fluoroquinolones, 1 quinolones, and 1 nitrofuran). We determined the frequencies of resistance and summarized the data for each isolate assayed against the 16 antibiotics (Table 11).

We performed Kirby-Bauer disk diffusion susceptibility tests [109] of c/t in triplicate on 993 ESBL+ *Enterobacteriaceae* isolates (899 *E. coli* and 94 *K. Pneumoniae*) from our collection. Isolated colonies were grown in Mueller Hinton broth for 16-18 hours at 37ºC, then plated as a lawn on Mueller Hinton II agar with c/t disk for 16-18 hours. The zone of inhibition (ZI) diameter was measured in millimeters and the ZI breakpoints for c/t were determined according to Clinical & Laboratory Standards Institute (CLSI) as follows: resistant: ZI ≤ 17 mm, intermediate: ZI = 18-20 mm, and susceptible: ZI ≥ 21 mm [109]. Additionally, we calculated empirical frequencies for multiple resistance to c/t and 16 other antibiotics as measured by DHMMC (Table 1).

We have positive PCR results for 852 isolates of the 993 for the presence of the four β-lactamase genes bla_{TEM}, bla_{OXA}, bla_{SHV}, and $bla_{\text{CTX-M}}$ [114]. Each PCR reaction consisted of 1 µL of template DNA, 10 µM of each primer, Taq 2X master mix (NEB) at a final concentration of 1X. The reactions were run under the following conditions: initial denaturation at 94°C for 10 min, 30 cycles of 94°C for 40 seconds, 60°C for 40 seconds, 72°C for 1 minute, and a final elongation at 72°C for 7 minutes. Multiplex PCR was used to determine the presence of $bla_{\text{CTX-M}}$, bla_{TEM}, and $bla_{\text{OXA,}}$ while bla_{SHV} was run in a separate reaction [115].

Statistical Analysis

We analyzed the ZI measurements for 852 clinical isolates in the presence of c/t as a function of four resistance genes. We performed ANOVA to assess differences among ZI measurements by resistance gene combinations using Tukey's honest significance test [116]. Analyses were performed using the Statistics and Machine Learning Toolbox of MATLAB R2020a.

Chapter 3: K. pneumoniae is more resistant to Ceftolozane/Tazobactam than E. coli

Abstract

Ceftolozane/tazobactam (c/t) is a relatively new antimicrobial therapy combination of a fifth-generation cephalosporin and inhibitor, approved in 2014 for treatment of complicated urinary tract infections (UTIs) and complicated intra-abdominal infections. Mechanisms of c/t resistance are still emerging and are not fully understood, prompting further study. Here, we analyzed the genomic sequences of ESBL-producing *Enterobacteriaceae* (*n* = 123) collected from hospitalized patients. We found preliminary association between *emrD*, *ompK35*, *ompK37*, and *ramR* with c/t resistance. PCR screening of these genes in 96 isolates did not show any significant associations. Additionally, we did not find significant associations between these four genes and the presence of bla_{TEM}, bla_{OXA}, bla_{SHV}, and $bla_{CTX\text{-}M}$ genes. Upon further investigations of different species with c/t resistance, we found that *K. pneumonia* is more associated with elevated resistance than *E. coli.* Although it is reassuring to know that c/t resistance is not due to a single gene, continuous investigations into mechanisms into its resistance are essential for the prolonged efficacy of this last resort drug therapy.

Introduction

Visits to physicians due to urinary tract infections (UTIs) surpass 7 million cases annually in the United States [117]. Complicated UTIs account for over 1 million hospital admissions yearly, with treatment being increasingly challenging due to rise in antimicrobial resistance [117,118]. Ceftolozane/tazobactam (c/t) is a new antimicrobial/inhibitor combination therapy recently approved in the United States for treatment of complicated UTIs and complicated intra-abdominal infections. Ceftolozane/tazobactam (c/t) is an intravenous cephalosporin combined with a β-lactamase inhibitor in a fixed 2:1 ratio [63,118]; ceftolozane inhibits cell-wall synthesis by binding to penicillin binding proteins, while tazobactam is a β-lactam that inhibits most of class A and some of class C β-lactamases [40,41,45,63]. This combination therapy was developed to address the rising rates of antimicrobial resistance in Gram-negative pathogens especially ESBL-producing *Enterobacteriaceae* [45,118].

Since its approval in 2014, resistance to c/t among *Enterobacteriaceae* has continued to be minimal globally [47,59,119] and in the agricultural area of Merced, CA[120]. Continuous surveillance and investigation into the causes of resistance, is essential for prolonging c/t efficacy. $bla_{CTX\text{-}M}$ and bla_{SHV} are β-lactamases that have been associated with elevated c/t resistance [40,55,61,108]. It has been shown that combinations of ESBLs and non-β-lactamase genes are associated with general β-lactam resistance [34,68,121–124]. Thus, the presence of $bla_{CTX\text{-}M}$ and bla_{SHV} on their own is not sufficient to drive the resistance phenotype observed in c/t [120,125] rather, there could be a combination of β-lactamases and non-β-lactamases that are also contributing to c/t resistance [125].

In a previous study, we examined the effects of β-lactamases on c/t resistance using clinical isolates mainly collected from patients with UTIs. We found that the presence of $bla_{CTX\text{-}M}$ and bla_{SHV}, although associated with resistance, is not insufficient to confer resistance on its own[120].

This led us to hypothesize that there may be non-β-lactamase genes helping confer c/t resistance. By looking at whole genomic sequences of a small sample of isolates (n = 123), we identified a group of candidate genes that are also involved in c/t resistance. These candidate genes include an efflux pump (*emrD*), porins (*ompK35*, *ompK37*), and an AcrAB efflux pump repressor (RamR). These four proteins affect membrane permeability and could be associated with β-lactam resistance [126]. To our knowledge, the association of these non-β-lactamase genes with c/t resistance has not yet been fully characterized and is currently conflicting. While a study found that the overexpression of AcrAB efflux pump decreased susceptibility to c/t [65], another study found that AcrAB did not have an impact on the activity of c/t [127]. Furthermore, other studies found that c/t was insensitive to efflux pumps and to reduced porin expression in *P. aeruginosa* [45], and to deficient OmpK35 in *Enterobacteriaceae* [127]. Castanheira et al. however, found that most c/t-non-susceptible *Enterobacteriaceae* isolates exhibited disrupted *ompK35* [128].

EmrD is an integral membrane transporter mainly found in *E. coli*. EmrD was originally identified as an efflux pump for uncoupler of oxidative phosphorylation that depletes the H^+ gradient, but then it was discovered to be a drug/H^+ antiporter [123]. EmrD is a potent antimicrobial efflux pump that prevents the accumulation of certain chemotherapeutics inside the cell, and have been associated with multidrug resistance in *Enterobacteriaceae* [123,129]. OmpK35 and OmpK37 are major porins found in the outer membrane of *Klebsiella pneumonia* and allow the diffusion and entry of a wide variety of molecules, including nutrients and antimicrobials [130]. The presence of OmpK35 is usually associated with β-lactam susceptibility as it facilitated the diffusion of antimicrobials to the periplasm, exposing the peptidoglycan[121,127], and the absence of a functional OmpK35 porin in ESBL-producing *Klebsiella* isolates contributes to increased β-lactam resistance [125,127,128]. OmpK37 was characterized more recently than other OmpKs, and was shown to have a narrower pore, thus intrinsically conferring increased resistance to β-lactam antibiotics by limiting diffusion into the periplasm [130,131].

RamR is a repressor of *RamA,* which is a global transcriptional activator of the *AcrAB* efflux pump [124,132]. *ramR* deficiency increases transcription of *ramA*, which in turn increases expression of the *AcrAB* efflux pump, contributing to multidrug resistance [124,132].

We used PCR to screen for the presence of these four genes in our collection of isolates, which includes many *Enterobacteriaceae* with c/t susceptibility that can be categorized as intermediate or resistant. We expect the presence of an efflux pump and/or an efflux pump regulator to be associated with resistance, while we expect the presence of the porins to contribute to susceptibility.

Results

Genomic sequence

The 123 sequenced isolates were mainly *E. coli* (*n* = 110), *K. pneumoniae* (*n* =11), and 3 other *Enterobacteriaceae*. We found the c/t resistance rate to be 8.33%; of the *E. coli* isolates, we found 7 resistant isolates, 55 with intermediate resistance, and 47 susceptible isolates. Of the *K. pneumonia* isolates, we found 3 resistant isolates, 5 with intermediate resistance, and 3 susceptible isolates. Although the c/t resistance rate of *E. coli* and *K. pneumoniae* is relatively low (8.33%), half of these sequenced isolates had intermediate c/t resistance (50%), and 41.67% of the isolates were susceptible.

Statistical analysis of genomic sequence data with the identified genes based on the Comprehensive Antibiotic Resistance Database (including partial matches) showed significant associations between c/t resistance and the two resistance genes OmpK35 ($p < 0.1$) and OmpK37 ($p < 0.1$) (Table S6) and their co-presence ($p = 0.054$) (Table S8). When we restricted the CARD gene search to only the best matches, we found that in these 123 isolates OmpK35 was present in 9 *K. pneumoniae* isolates (3 resistant, 3 intermediates, and 3 susceptible isolates). OmpK35, a *K. pneumonia* porin encoded in chromosomal DNA, usually contributes to β-lactam susceptibility, thus it is surprising to find that its presence is associated with c/t resistance.

Table 10. Isolates with OmpK35 from sequence data with average ZI measurements (mm). resistant: ZI ≤ 17mm, intermediate: ZI = 18-20mm, and susceptible: ZI ≥ 21mm

Sequenced samples with OmpK35	**species**	**Average ZI (mm)***
134	*K. pneumonia*	15.67
137	*K. pneumonia*	15.67
389	*K. pneumonia*	16
139	*K. pneumonia*	19
150	*K. pneumonia*	19
678	*K. pneumonia*	20
89	*K. pneumonia*	20.67
781	*K. pneumonia*	21.33
151	*K. pneumonia*	23.33

Sequence data indicated that *ompK37* was also associated with c/t resistance. Specifically, we found 3 *K. pneumoniae*, and one *E. coli* resistant isolates harboring *ompK37*. *ompK37* was also found 4 *E. coli* isolates with intermediate resistance and 1 susceptible isolate. In *K. pneumonia* we found 1 intermediate and 3 susceptible isolates harboring *ompK37* (Table 11). OmpK37 is normally associated with antibiotic resistance

due to the smaller channel in the porin, thus its association with c/t resistance is not surprising.

Table 10. Isolates with OmpK37 from sequence data with average ZI measurements (mm). resistant: ZI ≤ 17mm, intermediate: ZI = 18-20mm, and susceptible: ZI ≥ 21mm measurements (mm)

Sequenced samples with OmpK37	**Species**	**Average ZI (mm)**
750	*E. coli*	16.67
452	*E. coli*	19.33
388	*E. coli*	19.67
484	*E. coli*	20
96	*E. coli*	20.33
357	*E. coli*	20.33
87	*E. coli*	22.33
134	*K. pneumoniae*	15.67
137	*K. pneumoniae*	15.67
389	*K. pneumoniae*	16
678	*K. pneumoniae*	20
89	*K. pneumoniae*	20.67
781	*K. pneumoniae*	21.33
151	*K. pneumoniae*	23.33

Also from genomic sequence data, significant two-gene interactions between genes other than the porins were analyzed; *EmrD* and *ramR* presence in the same isolates showed significant association with c/t resistance ($p < 0.1$) (Table S7). Other significant co-occurrences with emrD were omitted due to their ubiquitous presence with genes other than emrD. Distribution of these non-β-lactamase enzymes in the PCR screened isolates is represented in Figure S6.

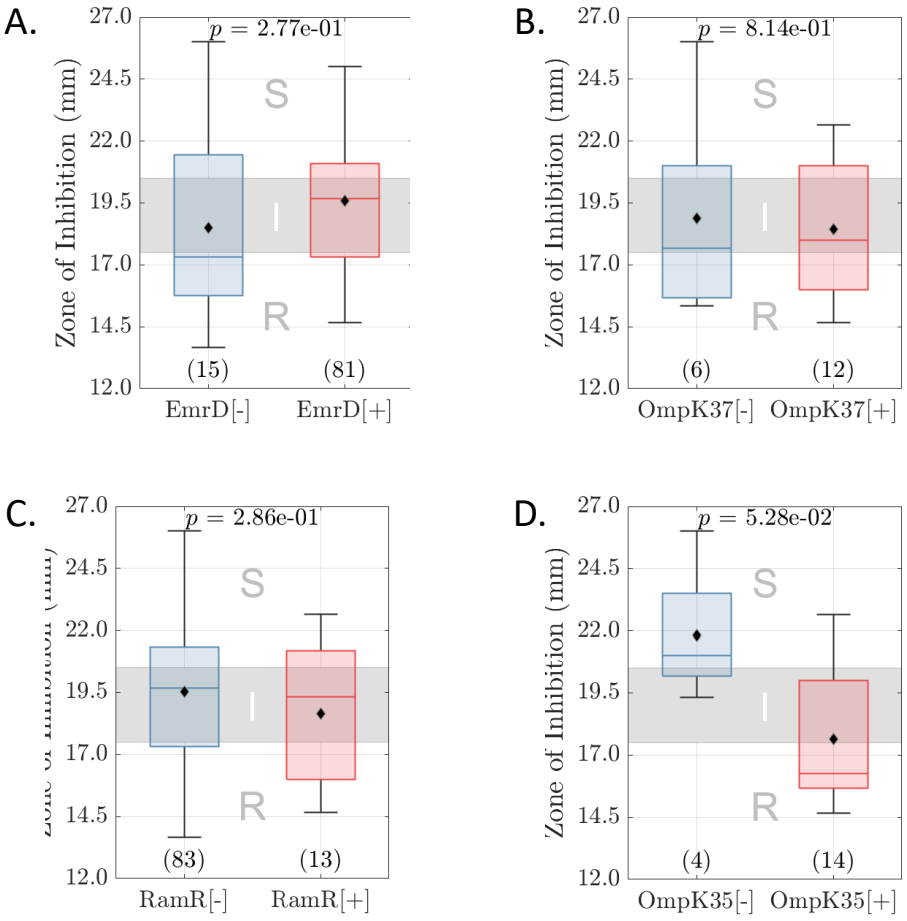
Polymerase Chain Reaction

PCR data of 96 samples that screened for *emrD*, *ramR*, *OmpK35*, and *OmpK37* showed no significant associations between their presence/absence and c/t resistance (Figure 9, and Table S10). Additionally, we did not find significant interactions between the presence of these non-β-lactamase genes in the PCR screen and the β-lactamase genes previously studied, after performing the FDR controlling procedure (Table S10). Distribution of β-lactamase genes in PCR screened isolates is represented in Figure S7.

Looking for species differences with c/t resistance, we found that *E. coli isolates are more susceptible* to c/t than *K. pneumoniae*, though this association is not statistically

significant (p = 0.17). These results are not surprising as other studies have found *K. pneumoniae* to be more resistant than *E. coli.*

Figure 9. Presence ([+]) and absence ([-]) of four non-β-lactamase resistance genes and their association with c/t resistance. S = susceptible, I = intermediate, R = resistant. Diamond (⧫) = mean. A. RamR presence ([+]) and absence ([-]). B. EmrD presence ([+]) and absence ([-]). C. OmpK37 deficiency presence ([+]) and absence ([-]). D. OmpK35 deficiency presence ([+]) and absence ([-]). *p* value is based on a one-sided Welch's t-test.



Discussion

Resistance to β-lactam antibiotics is an ongoing battle that jeopardizes bacterial infection treatment. Pathogenic ESBL-producing *Enterobacteriaceae* continue to evolve and acquire different means of resistance, including the production of β-lactamases that hydrolyze the antibiotic and/or other means to evade it such as efflux pumps. Here, we characterized two non-β-lactamase mechanisms of resistance to c/t, a last resort antibiotic [22]. Genomic sequence analysis gave us a large set of resistance genes (n = 183), of which a narrow set was selected based on limited interactions with other genes and biological significance. The presence of *ompK35* and *ompk37* were significantly associated with c/t resistance and considered for further confirmation by PCR, along with *emrD*, and *ramR* for a combined effect screening.

Our genomic sequence data and PCR screening data showed an association between the presence of *ompK35* and c/t resistance. These results are surprising since having a functional *ompK35* is usually associated with β-lactam susceptibility [121,125,127,128]. While we do not understand the mechanism by which *ompK35* is associate with c/t resistance, at the very least its presence is not associated with c/t susceptibility, which is a behavior that is different from most cephalosporins [121]. We PCR screened for the presence of the porins along with efflux pump and efflux pump regulator genes. Upon investigation the PCR data, all statistically significant associations were lost. We initially found a significant association between the presence of *ompK35* and c/t resistance, but that is when we looked at all *Enterobacteriaceae*. When we separated the isolates by species, we lost those significant interactions.

We then tested for associations between c/t resistance and species and found *K. pneumoniae* to be more associated with c/t resistance than *E. coli.* Other studies have found similar results, confirming our results [58,59].

In a previous study[120], we looked at associations of β-lactamase genes with c/t resistance and found the $bla_{\text{CTX-M}}$ and bla_{SHV} showed such associations. We looked at associations between these β-lactamase genes and the non- β-lactamase tested here. While we did not observe a statistically significant combined beneficial effect of having CTX-Ms/SHVs with non-ESBL genes on c/t resistance after performing FDR controlling procedure, two studies have observed such effects [127,128]. Nicolas-Chanoine et al., found that CTX-M-15 producing *Klebsiella* strains became non-susceptible to c/t with Ompk35 porin loss [127], and Castanheira et al., found that among the c/t non-susceptible *Klebsiella* isolates with disrupted *ompK35*, many had elevated expression of $bla_{\text{CTX-M}}$ [128].

In conclusion, it seems that resistance to c/t is more complex as there is no single resistance gene that has a large effect on c/t resistance. We studied the effects of combinations of 8 common ESBLs and non-ESBL genes with c/t resistance and concluded

that not 1 single gene by itself is responsible for the resistance. C/t resistance is a complex phenotype with complex underlying genotype.

Methods and Materials

Ceftolozane/Tazobactam Kirby-Bauer Disk Diffusion

Dignity Health Mercy Medical Center (DHMMC) in Merced, CA provided us with over 1,200 ESBL-producing isolates (ESBL+) associated mainly with urinary tract infections. Along with the isolates to add to our repository, they included the resistance profile to 16 commonly used antibiotics based on MIC data from the VITK2 [110,120]. From the DHMMC collection, 858 frozen samples were streaked on agar plates. Single colonies were then transferred to 2 ml Mueller Hinton broth and incubated in oxygen limiting conditions for 20 hours at 37ºC. 100 μM of the broth culture was then transferred to Mueller Hinton II agar and spread with glass beads. Plates were stamped with ceftolozane (30 μg)/tazobactam (10 μg) and incubated for 17 hours at 37ºC. The Zone on Inhibition (ZI) was measured in millimeters. The CLSI breakpoints for c/t are as follows: resistant: ZI ≤ 17 mm, intermediate: ZI = 18-20 mm, and susceptible: ZI ≥ 21 mm [109]. Three replicate tests for each sample were performed, and the average ZI was used for the analysis[120].

Sequencing

Genomic DNA was extracted from 123 DHMMC sample using the ZR-96 Quick-DNA Kit from Zymo Research at the University of California, Merced. Whole-genome sequencing including TruSeq DNA library preparation was performed at the University of California, Davis Genome Center using Illumina's MiSeq and HiSeq technologies, and at the University of California, Berkeley using HiSeq. We obtained 24 MiSeq (2 x 250 bp) and 109 HiSeq (2 x 250 bp) paired-end sequencing libraries. Prior to assembly, FASTq sequences (raw reads) were concatenated, then low quality bases were trimmed from the sequencing reads using Sickle Master (version 1.33) [133]; trimmed reads shorter than 36 bp were discarded. De novo paired-end assembly was conducted for each MiSeq and HiSeq library using SPAdes (version 3.11.1) [134] with read error correction by BWA-spades. The assemblies had 29x and 327x median coverage for MiSeq and HiSeq libraries, respectively. Resistance genes (RE) from NCBI's Comprehensive Antibiotic Resistance Database (CARD) [135] were used to create an RE library; using BLASTn, FASTA files were blasted against the RE library with the maximum target match. The sequenced collection had 111 *E. coli*, 10 *K. pneumoniae*, 2 other *Enterobacteriacea*e.

Statistical methods

We wanted to statistically determine which of 183 resistance genes identified by CARD were associated with c/t resistance. We analyzed 123 clinical isolates for which we had the c/t ZI measurements (mm), and the genetic information (presence/absence) of 183 resistance genes.

Genes that appeared with too low frequency (less than 8/123) or too high frequency (115/123) within our isolate population were not considered informative and were omitted. Using the 77 remaining genes, we performed a Welch's t-test between the ZI measurements and presence vs. absence of each gene. We also considered the possibility of combined effects between markers and the resulting ZI measurements. From this test, genes whose presence were associated with a decrease in ZI measurement ($p < 0.1$) and were biologically relevant to resistance were considered for further study. *The ompK35* and *ompK37* genes showed significant association with c/t resistance independently and were biologically relevant. OmpK35 increases cephalosporin susceptibility, while OmpK37 increases cephalosporin resistance. We wanted to see if the absence of *OmpK35* was associated with c/t resistance, and if the presence of *OmpK37* was associated with resistance through PCR. Two-gene interactions were then considered. Many interactions were excluded due to ubiquitous interactions with almost all other genes. We reasoned that the effects of genes interacting with several other resistance determinants would be difficult to assess with any certainty because of the unknown genomic variation present in our collection. We did find a subset of resistance genes with fewer interactions. There was statistical significance with *emrD* interacting with 3 other non-β-lactamase genes (*ramR*, *ompK35*, and *ompK37*) which did not appear to interact with many other resistance determinants; and have been associated with β-lactam resistance [65,123,124,132]. Therefor we selected these 4 candidate genes (*emrD*, *ramR*, *ompK35*, and *Ompk37*) for PCR screening to add non-β-lactamase gene information to our consideration of c/t resistance.

From the ZI measurement, we determined that 32 isolates are resistant, 314 are intermediate, and 512 are susceptible to c/t[120]. In order to create a balanced dataset, we opted to select the 32 resistant isolates, and uniformly at random selected 32 from the 314 isolates with intermediate resistance, and similarly selected 32 from the 512 susceptible isolate set, for a representative dataset of 96 isolates. Using the PCR information of the four resistance genes on our representative dataset of 96 isolates, we performed a Welch's t-test on the ZI measurements for these four markers considering their presence vs absence.

We also considered the possibility of nonlinear interactions between these four genes and the presence of β-lactamase CTX-M, TEM, SHV, and OXA[120] using ANOVA (Tukey's Honestly Significant Differences test). In the study for nonlinear interactions, only 95 isolates were used as there was PCR data missing for one isolate in the β-lactamase set, thus we omitted it in the ANOVA analysis. We controlled for multiple statistical tests using the False Discovery Rate (FDR) controlling procedure [136], with a false discovery control level of q*=0.05. We only report as significant those results that remained significant after the FDR controlling procedure.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) was performed on the 96 isolates (32 resistant, 32 intermediate, 32 susceptible) described above. The primers used were as follows:

Gene	Forward	Reverse
emrD	5' ATCTCAACGTCCGTGAAGGG3'	3'AAACAGCTGTGAGACACCGT5'
ramR	5'CAGCTATATCGACTGGGGCG3'	3'TTCAAAGCCGAGGGCGATAA5'

OmpK35	5’TTCTTCGGTCTGGTTGACGG3’	3’CGCTACGGTTAGAGCTGGAG5’
OmpK37	5’AAAAACGAAGGCCAGAACGC3’	3’TGCCTTTGGACTGCAGGTAG5’

Each PCR reaction consisted of 1 µL of template DNA, 10 µM of each primer, Taq 2X master mix (NEB) at a final concentration of 1X, and the reactions were run under the following conditions, initial denaturation at 94°C for 10 min, 30 cycles of: 94°C for 40 seconds, 61°C for 40 seconds, 72°C for 1 minute; and a final elongation at 72°C for 7 minutes. *ramR*, *ompK35*, and *ompK37* were multiplexed in the same reaction, while *emrD* was amplified in a separate reaction.

Chapter 4: Look who's talking: instructor and discourse practices across discipline, position, experience, and class size in STEM college classrooms

Abstract

Students are more likely to learn in college science, technology, engineering, and math (STEM) classrooms when instructors use teacher discourse moves (TDMs) to encourage engagement and learning. However, while teaching practices are well studied, TDMs are not well understood in college STEM classrooms. In STEM courses at a Hispanic-Serving Institution (HSI) ($N = 74$), we used two classroom observation protocols to investigate teaching practices and TDMs across disciplines, instructor types, years of teaching experiences, and class sizes. We found biology instructors *guide* students in active learning activities but use *authoritative* discourse approaches. Additionally, chemistry instructors *presented* more than instructors teaching other disciplines. Also, teaching faculty had high *dialogic, interactive* discourse; and neither years of faculty teaching experience or class size had an impact on instructional or discourse practices. Our results indicate differences in classroom practices across STEM disciplines and instructor types with implications for targeted teaching professional development efforts.

Introduction

The Classroom Observation Protocol for Undergraduate STEM (COPUS) and the Classroom Discourse Observation Protocol (CDOP) are classroom observation tools that allow researchers to assess teaching and discourse practices [88,97]. A previous study combining COPUS and CDOP showed that it is possible to create a classroom environment with high student-centered, evidence-based teaching practices but with low *dialogic, interactive* teacher discourse moves. This indicates that even when instructors are engaging, they are still teaching with teacher-centered discourse pedagogies. However, this previous work only examined biology instructors' classroom teaching practices in mostly introductory undergraduate biology classes at a research-intensive, predominantly white institution [97]. Therefore, building upon recent work Kranzfelder et al. (2020) in biology classrooms, we wanted to expand and examine teaching and discourse practices across a range of Science, Technology, Engineering, and Mathematics (STEM) instructors and course characteristics, such as in different disciplines, instructor types, years of teaching experiences, and class sizes at a research-intensive, Hispanic-Serving Institution (HSI).

Instructors play a key role in facilitating student engagement through enacted classroom discourse

Students are more likely to learn in college STEM classrooms when encouraged to analyze and challenge questions and work collaboratively in small groups to answer instructors' questions [137–139]. Therefore, instructors play a unique role in facilitating student engagement through deliberate actions taken to mediate, participate in, or influence classroom discourse or the verbal instructor-student and student-student interactions used to construct meaning [140–142]. One type of classroom discourse, *teacher discourse moves* (TDMs), are the conversational strategies used by instructors to support student understanding of content knowledge [97,143] and have been found to foster student learning by engaging students in a deeper understanding of the scientific ideas [144–146]. In 2015, Seidel and colleagues coined the term *Instructor Talk* to describe the non-content related conversational language used by instructors [147]. An example of *Instructor Talk* would be when an instructor gives instructions for classroom activities or justifications for active learning use. While this type of discourse facilitates overall learning in the classroom, it is different from the content-related discourse that we refer to here as TDMs.

Prior work assessing TDMs in primary and secondary STEM classrooms found that the Initiate-Response-Evaluate (IRE) discourse pattern that focuses on fixed communication was the prevailing form of dialogue between instructors and students [148,149]. An example of IRE would be an instructor asking a yes or no question (initiate), receiving a yes or no answer (response), and confirming that answer as either correct or incorrect (evaluate). However, the less frequently occurring Initiate-Response-Feedback (IRF) discourse pattern creates opportunities for student-instructor dialogue by generating collaborative discussions. An example of IRF would be an instructor asking a question (initiate), receiving an answer (response), and then prompting the student for follow-up dialogue (feedback). It is thought that IRF discourse approaches are more effective than IRE in promoting student discussions as they create opportunities for students to develop critical reasoning and argumentation [150,151]. More recently, in undergraduate biology classrooms, Kranzfelder et al. (2020) found that the IRE discourse pattern was the most dominant form even when instructors were teaching with student-centered, active learning strategies.

Classroom Observation Protocols

Commonly, classroom observation protocols are used to study and improve STEM teaching practices by providing research-supported recommendations [152]. In contrast to surveys or interviews, well-developed, reliable classroom observations provide a third-party impartial way of delivering targeted feedback to the instructor [153]. This impartiality is in part due to predisposed biases held by students filling out surveys or answering interview questions as opposed to a trained third-party making observations [154,155]. Many classroom observation protocols have been developed, including the Practical Observation Rubric To Assess Active Learning (PORTAAL) [86], the Decibel Analysis for Research in Teaching (DART) [87], the Classroom Observation Protocol for Undergraduate STEM (COPUS) [88], the Reformed Teaching Observation Protocol (RTOP) [89], and the Classroom Discourse Observation Protocol (CDOP) [90].

There are differences in how each of these classroom observation protocols characterizes instructional practices. Briefly, PORTAAL is intended to support STEM instructors moving from instructor-centered to active learning-based instruction [86], while DART analyzes the volume and variance of classroom sound to accurately predict the learning activities used in classrooms [87]. RTOP provides a standardized means to determine the degree of student-centered and engaged

learning practices using a 5-point Likert scale for 25 items [89]. Moreover, RTOP requires multiday training for acceptable interrater reliability, and it can be problematic and challenging to share RTOP observational judgments with the observed instructor. However, COPUS is a non-evaluative classroom observation protocol that provides an objective account of what both instructors and students are doing during a class period [88]. Tools like COPUS and RTOP examine the prevalence of instructional practices without assessing TDMs, whereas CDOP measures TDMs [90].

A combination of two classroom observation protocols, COPUS [88] and CDOP [90], have been found to provide a holistic view into college STEM classrooms [97]. COPUS documents instructional practices in two-minute intervals throughout the entire class session with 25 codes in two categories: 1) what the instructor is doing and 2) what the students are doing. The 25 codes correspond to 12 instructional behaviors, such as lecturing, posing and answering questions, individual thinking, different kinds of classroom discussions, and 13 student behaviors, such as listening to lecture or posing questions to the instructor [88]. COPUS can be used to analyze patterns in instructional practices, observe what codes instructors commonly pair together, and examine how instructional practices may differ among instructors with different characteristics [95,96,156]. In contrast, CDOP documents discourse practices, specifically TDMs, in two-minute intervals throughout the entire class session with 17 individual codes and four collapsed codes. The four codes categorize instructor discourse by determining if there are authoritative or dialogic teacher-initiated interactions and if they include interactions with the students or are teacher-centered [90]. More specifically, CDOP adapted the analytic framework developed by Morimer and Scott (2005) into four discourse patterns as described here:

(1) **Authoritative, Non-Interactive** is classroom discourse where the instructor focuses on their point of view with no student participation opportunities (e.g., lecturing).
(2) **Authoritative, Interactive** is classroom discourse where the instructor is the main participant but leads students through a question-and-answer routine to consolidate their point of view (e.g., lecturing with IRE-type questions).
(3) **Dialogic, Interactive** is classroom discourse where both the instructor and students participate. Here, the instructor listens and responds to student discourse, and students benefit from teaching guidance (e.g., whole-class discussion with IRF-type questions).
(4) **Other** is if a TDM was observed, but no identifiable codes fit.

Instructor and course characteristics that might impact instructional and discourse practices

Kranzfelder et al. (2020) showed that even when instructors mostly implemented student-centered, active learning teaching practices, they were not always paired with student-centered TDMs. However, that study had limitations as it only examined the classroom practices of biology instructors teaching in mostly introductory undergraduate biology classes. Thus, it is essential to investigate teaching practices using COPUS and discourse practices using CDOP across different instructor and course characteristics, including STEM disciplines, instructor types, years of faculty teaching experience, and class size, to expand on previous research and broaden our understanding of what is happening in college STEM classrooms.

Prior studies have found differences in teaching practices across STEM disciplines [78,94,95]. First, Lund et al. (2015) found that chemistry instructors *lectured* disproportionately more than biology instructors, while biology instructors implemented more *peer instruction*, and mathematics used more *collaborative learning*. Additionally, they found that chemistry, physics, and engineering courses are most often taught through *lecturing* [94]. More recently, Eagan (2016) found that different STEM disciplines, such as biology or mathematics, incorporate other course materials per their subjects and use different scientific inquiry approaches in their courses [78]. These differences in implementation affect student performance outcomes and learning gains. They also found that math and engineering consistently used fewer electronic quizzes with immediate feedback and student inquiry to drive learning compared to biology [78]. And finally, Stains et al. (2018) found mathematics instructors to use more student-centered styles, while biology instructors have more interactive teaching styles, and chemistry instructors use more *didactic* styles (*didactic* is 80% or more of class time consists of *lecturing*) [95]. These studies suggest that different STEM disciplines have different cultures of implementing student-centered, evidence-based teaching practices in their courses.

Additionally, instructors' academic positions or instructor types have also been shown to influence instructional practices [98,157,158]. For example, in the University of California system, there are three main instructor types: tenure-track research faculty, tenure-track teaching faculty (also known as Lecturer with Potential Security of Employment (L(P)SOE)), and non-tenure-track lecturer (also known as contingent faculty, part-time, or Unit-18 lecturer). Each of these instructor types include widely different expectations for research, teaching, and service and opportunities for teaching professional development. For example, tenure-track research faculty are primarily evaluated on the success of their research programs [159], and their teaching is generally not an important area for advancement [160]. In contrast, tenure-track teaching faculty are expected to spend more time preparing for their classroom instruction and to be more knowledgeable about evidence-based teaching practices [158]. Finally, lecturers are the predominant instructor type in higher education with teaching expectations, but not research or service [161]. Also, when comparing tenure-track teaching faculty to lecturers, tenure-track teaching faculty tend to have more opportunities for teaching professional development and a smaller teaching load than that of lecturers who often teach up to 5 courses per semester [162]. It has been well documented that discipline-based professional development improves undergraduate STEM classroom outcomes [163,164] and promotes opportunities for faculty to learn about alternative approaches to teaching [165].

A third instructor characteristic that might impact teaching and discourse practices is years of faculty teaching experience. It has been shown that novice teachers hold simplistic views on teaching and learning [166]. Therefore, they are most likely not incorporating evidence-based teaching practices into their classrooms. With experience comes a better understanding of classroom management, indicating better communication with students [167]. It has been shown that the level of student-centeredness increases as instructional experience increases [94]. Additionally, different class sizes dictate how instructors can implement certain activities and interact with students. For example, in a small class with less than ten students, instructors can learn all the students' names and become familiar with each student individually. However, in large lecture courses with over 300 students, this becomes nearly impossible, and instructors may resort to traditional lecturing instead of varying their discourse patterns. Even in flexible classroom layouts and small course

sizes, there is not always an increase in student-centered practices [95]. Finally, Smith et al. (2014) found a significant positive correlation between the percentage of *presenting* as measured by COPUS and class size (Pearson's $r = 0.401$, $p < 0.05$), indicating that instructors who teach large-enrollment classes tend to *present* more often [93].

Building upon previous work in biology classrooms from Kranzfelder et al. (2020), we wanted to test if these results hold in *all* STEM classrooms at a research-intensive HSI. Specifically, we asked the following three questions:

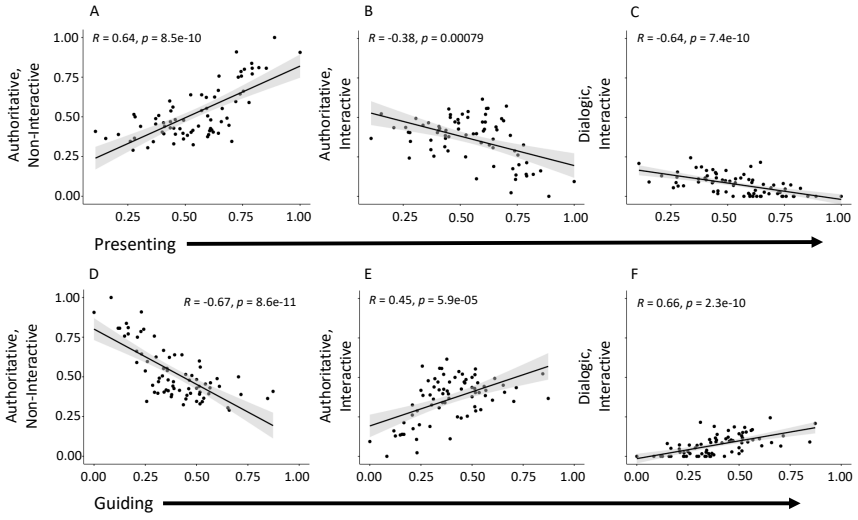
(1) How do instructional practices correlate with discourse practices?
(2) Are there differences in instructional practices and discourse practices used by STEM instructors?
(3) Are there differences in instructional and discourse practices across various instructor and course characteristics, including STEM disciplines, instructor types, years of teaching experience, and class size?

Results

Correlations between Instructional (COPUS) and Discourse Practices (CDOP) Used by STEM Instructors

We correlated two COPUS collapsed codes to three CDOP collapsed codes and found significant associations between all six pairs of variables ($p < 0.001$) (Figure 10, Tables S22 and S23). In particular, we found that *presenting* positively correlated with *authoritative, non-interactive* ($\rho = 0.64$, Figure 10A), but negatively correlated with *authoritative, interactive* ($\rho = -0.38$, Figure 10B) and *dialogic, interactive* ($\rho = -0.64$, Figure 10C). In contrast, *guiding* negatively correlated with *authoritative, non-interactive* ($\rho = -0.67$, Figure 10D), but positively correlated with *authoritative, interactive* ($\rho = 0.45$, Figure 10E) and *dialogic, interactive* ($\rho = 0.66$, Figure 10F). This suggests that *presenting* teaching practices and *authoritative, non-interactive* discourse practices were commonly implemented together while *guiding* and *authoritative, interactive* and *dialogic, interactive* were commonly implemented together (Figure 10).

Figure 10. Three discourse approaches (i.e., authoritative, non-interactive; authoritative, interactive; and dialogic, interactive) in response to instructional practices (i.e., presenting and guiding). Scatter plots are shown with Spearman's correlation coefficient (ρ) and p-value (*p*) for (A) presenting versus authoritative, non-interactive; (B) presenting versus authoritative, interactive; (C) presenting versus dialogic, interactive; (D) guiding versus authoritative, non-interactive; (E) guiding versus authoritative, interactive; and (F) guiding versus dialogic, interactive



Broad Instructional Practices Used by STEM Instructors (COPUS)

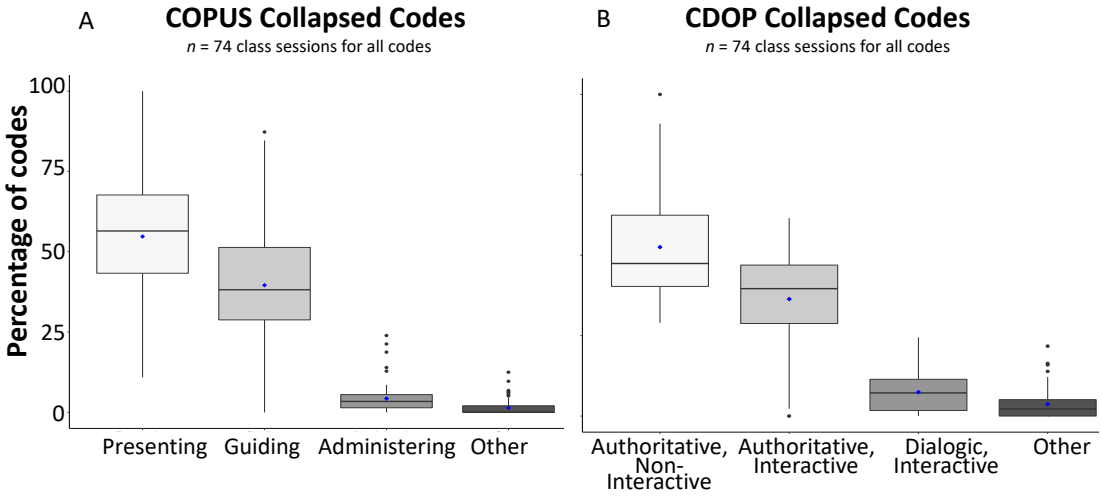
We used COPUS collapsed codes to quantify broad instructional practices of our STEM instructors and found that they were mainly *presenting* information to students (e.g., *lecturing*) but

also *guiding* students in active learning activities (e.g., moving around and facilitating small group or whole-class discussion) (χ^2 = 189, df = 3 p < 0.001, W = 0.85). More specifically, STEM instructors were spending significantly more of their class time *presenting* information to students (m = 54.7%, range of 10.9% to 100% across all class sessions) than *guiding* students in active learning activities (m = 39.6%, range of 0% to 87.3% across all class sessions). Finally, STEM instructors were spending significantly less class time *administering* (m = 4.3%) and *other* instructional practices (m = 1.4%) (Figure 11A, Table S18 and S19).

Broad Discourse Practices Used by STEM instructors (CDOP)

We used CDOP collapsed codes to quantify the broad discourse practices of our STEM instructors and found that they were mainly using *authoritative* discourse approaches (i.e., only lecturing or lecturing with IRE-type questions) and spent significantly less time on *dialogic* discourse approaches (i.e., the instructor asks students to talk about content) (χ^2 = 175, df = 3, p < 0.001, W = 0.79). For example, *authoritative* discourse practices were eleven times more likely to occur than dialogic ones. More specifically, STEM instructors spent significantly more of their class time using *authoritative, non-interactive* discourse practices (m = 52.5%, range of 29.0% to 100% across all class sessions) compared to *authoritative, interactive* discourse practices (m = 36.4%, range of 0.0% to 61.5% across all class sessions), *dialogic, interactive* discourse practices (m = 7.4%, range of 0% to 24.4% across all class sessions), and *other* (i.e., *no content discourse*) discourse practices (m = 3.7%, range of 0% to 21.7% across all class sessions) (Figure 11B, Table S20 and S21).

Figure 11. Box-and-whisker plots showing the percentage of codes that instructors spent on different instructional practices (A) and discourse practices (B) across 74 STEM class sessions. The boxes represent the interquartile range (IQR) of practices for each collapsed code, whiskers represent the largest and smallest values within 1.5 times the IQR, lines within each box represent the median, the blue diamond represents the mean, and the black dot represents the outliers.



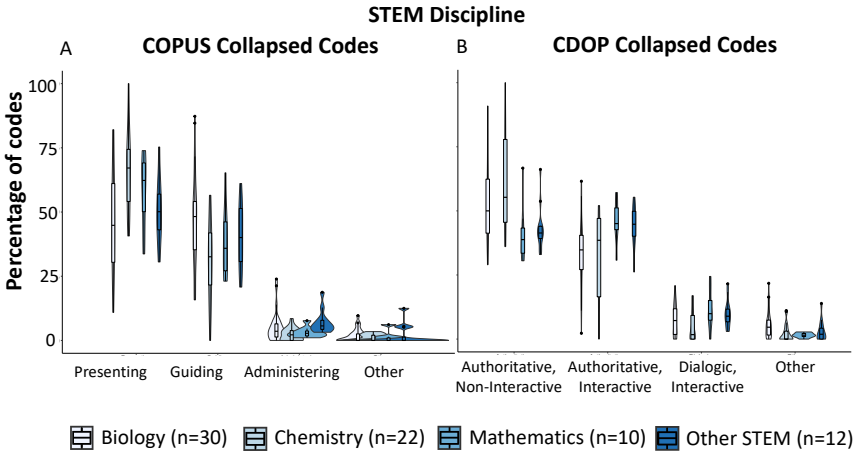
Instructional (COPUS) and discourse (CDOP) practices across STEM disciplines

We found significant differences in the instructional practices across STEM disciplines ($F(9, 280) = 4.85$, $p < 0.001$, $\eta p^2 = 0.13$). Looking at individual STEM disciplines and COPUS codes, we found that biology instructors *presented* a mean of 47.1% with a range of 10.9-82.1% and *guided* a mean of 46.3% with a range of 15.8-87.3%. Chemistry instructors *presented* a mean of 65.9% with a range of 40.7-100% and *guided* a mean of 30.7% with a range of 0-56.5%. Mathematic instructors *presented* a mean of 58.1% with a range of 33.7-73.9% and *guided* a mean of 37.9% with a range of 23.1-65.3%. Other STEM instructors *presented* a mean of 50.5*%* with a range of 30.6-75.3% and *guided* a mean of 40.5% with a range of 20.8-61.1%. Overall, we found that chemistry instructors used significantly more *presenting* than biology ($p < 0.001$) and other STEM ($p < 0.001$) instructors, while chemistry instructors used significantly less *guiding* than biology ($p > 0.001$) and other STEM ($p = 0.04$) instructors (Figure 12A, Tables S24, and S25).

Similarly, we found significant differences in discourse practices across STEM disciplines ($F(9, 280) = 3.25$, $p < 0.001$, $\eta p^2 = 0.09$). Looking at individual STEM disciplines and CDOP codes, we found that biology instructors used *authoritative, non-interactive* a mean of 51.0% with a range of 29.0-83.7%, *authoritative, interactive* a mean of 36.24% with a range of 11.1-61.5%, and *dialogic, interactive* a mean of 7.4% with a range of 0-21.8%. Chemistry instructors used *authoritative, non-interactive,* a mean of 60.7% with a range of 36.1-100%, *authoritative, interactive,* a mean of 32.3% with a range of 0-52.1%, and *dialogic, interactive* a mean of 4.7% with a range 0-16.9%. Mathematics instructors used *authoritative, non-interactive,* a mean of 49.6% with a arrange of 30.5-90.9%, *authoritative, interactive,* a mean of 37.7% with a range of 2.3-57.1%, and *dialogic, interactive* a mean of 10.0% with a range of 0-24.4. Other STEM disciplines used *authoritative, non-interactive* a mean of 43.7% with a arrange of 33.0-66.2%, *authoritative, interactive* a mean of 43.0% with a range of 26.2-55.3%, and *dialogic, interactive* a mean of 10.4% with a range 3.0-21.6% (Figure 12B, Tables S26, and S27).

Overall, we found that chemistry instructors used significantly more *authoritative, non-interactive* discourse approaches than biology ($p = 0.04$) and other STEM disciplines ($p < 0.001$). However, chemistry instructors used significantly less *authoritative, interactive* and *dialogic, interactive* discourse approaches than other STEM disciplines ($p = 0.02$). Biology instructors used significantly more *authoritative, interactive* than other STEM instructors ($p = 0.03$). Additionally, Other STEM instructors used significantly more *dialogic, interactive* than chemistry instructors ($p = 0.02$), while we did not find significant differences in the usage of *dialogic, interactive* between biology, chemistry, and mathematics. There were also no statistically significant differences between STEM disciplines for *administering* for COPUS and *other* for CDOP (Figure 12B, Tables S24, S25, S26, and S27).

Figure 12. Violin and box-and-whiskers plots show the percentage of codes that instructors spent on different instructional practices (A) and discourse practices (B) across STEM disciplines, including biology, chemistry, mathematics, and other STEM. The violin represents the density of the code frequency. The boxes represent the interquartile range (IQR) of practices for each collapsed code, whiskers represent the largest and smallest values within 1.5 times the IQR, lines within each box represent the median, and the black dot represents the outliers.



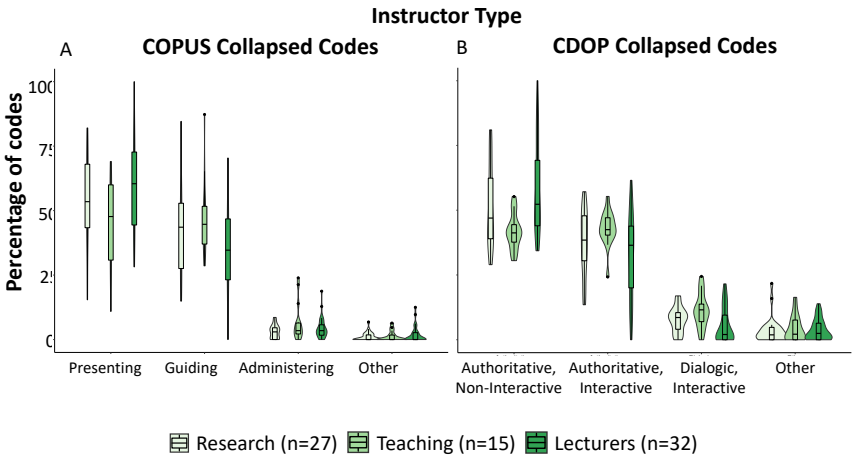
Instructional (COPUS) and discourse (CDOP) practices across instructor types

We found significant differences in the teaching practices (collapsed COPUS codes) across instructor types ($F(6, 284) = 2.48$, $p = 0.02$, $\eta p^2 = 0.05$). We found that research faculty *presented* a mean of 54.1% with a range of 15.4-82.1%, while they *guided* a mean of 41.8% with a range of 14.9-84.6%. Teaching faculty *presented* a mean of 45.6% with a range of 10.9-69.1%, while they *guided* a mean of 46.7% with a range of 28.6-87.3%. And finally, lecturers *presented* a mean of 59.6% with a range of 28.2-100%, while they *guided* a mean of 34.4% with a range of 0-70.4%. Overall, we found that teaching faculty used significantly less *presenting* than lecturers ($p = 0.0$), but not research faculty ($p = 0.19$); while teaching faculty used significantly more *guiding* than lecturers ($p = 0.01$), but not research faculty ($p = 0.34$) (Figure 13A, Tables S28 and S29).

Similarly, we found significant differences between instructor type and discourse practices (collapsed CDOP codes) on the percentage of codes ($F(6, 284) = 5.554$, $p < 0.001$, $\eta p^2 = 0.11$). We found that research faculty used *authoritative, non-interactive* 51.5% with a range of 29.0-81.0%, *authoritative, interactive* a mean of 37.2% with a range of 13.5-57.1%, and *dialogic, interactive* a mean of 7.8% with a range of 0-17.0%. Teaching faculty used *authoritative, non-interactive* 41.7% with a range of 30.5-55.3%, *authoritative, interactive* a mean of 42.9% with a range of 24.2-55.3%, and *dialogic, interactive* a mean of 11.2% with a range of 0-24.4%. Lecturers used *authoritative, non-interactive* 58.4% with a range of 34.4-100%, *authoritative, interactive* a mean of 32.6% with a range of 0-61.5%, and *dialogic, interactive* a mean of 5.4% with a range of 0-21.6%. Overall, teaching faculty used significantly less *authoritative, non-interactive* than lecturers ($p < 0.001$) and research faculty ($p = 0.01$); while teaching faculty used significantly more *authoritative, interactive* than lecturers ($p < 0.001$) and research faculty ($p = 0.03$); teaching faculty used significantly more *dialogic, interactive* than lecturers ($p = 0.01$). We found no significant differences between teaching

faculty and research faculty for *dialogic, interactive* (p = 0.21). There were no statistically significant differences between instructor types for *administering* and *other*. We interpret this to mean that both teaching faculty and research faculty use *guiding* instructional practices and *dialogic, interactive* discourse, but teaching faculty also use *authoritative, interactive* discourse more than the two other instructor types (Figure 13B, Tables S30, and S31).

Figure 13. Violin and box-and-whisker plots showing the percentage of codes used by instructors' types for instructional practices (A) and discourse practices (B). The violin represents the density of the code frequency. The boxes represent the interquartile range (IQR) of practices for each collapsed code, whiskers represent the largest and smallest values within 1.5 times the IQR, lines within each box represent the median, and the black dot represents the outliers.



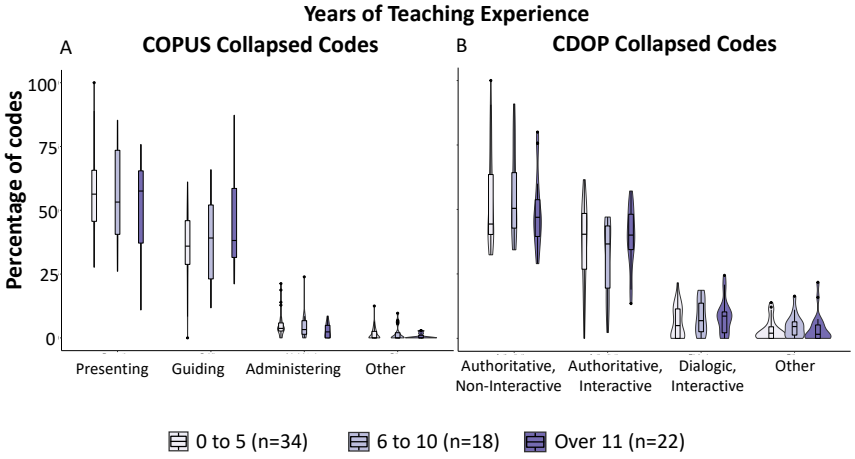
Instructional (COPUS) and discourse (CDOP) practices across years of faculty teaching experience

We did not find significant differences between years of faculty teaching experience and collapsed COPUS codes on the percentage of codes ($F(6, 284) = 0.76$, $p = 0.6$, $\eta p^2 = 0.05$). We found that instructors with 0-5 teaching experience *presented* a mean of 57.6% with a range of 27.7-100% and *guided* a mean of 35.7% and a range of 0.0-61.1%. Instructors with 6-10 years of teaching *presented* a mean of 54.8% with a range of 26.1-85.3%. Instructors with 11 years or more of teaching experience *presented* a mean of 50.2% with a range of 10.9-75.8% and *guided* a mean of 46.4% with a range of 21.2-87.3% (Figure 14A, Tables S32, and S33).

Similarly, for CDOP, we did not find significant differences between years of faculty teaching experience and collapsed CDOP codes on the percentage of codes ($F(6, 284) = 1.06$ p = 0.38, $\eta p^2 = 0.02$). Instructors with 0-5 years of teaching experience used *authoritative, non-interactive* discourse a mean of 53.4% with a range of 32.4-100%, *authoritative, interactive* a mean of 37.6% with a range of 0.0-61.5%, and *dialogic, interactive* a mean of 6.4% with a range of 0.0-21.6%. Instructors with 6-10 years of teaching experience used *authoritative, non-interactive* discourse a mean of 55.4% with a range of 34.4-90.9%, *authoritative, interactive* a mean of 31.7% with a range of 2.3-47.1%, and *dialogic, interactive* a mean of 8.3% with a range of 0-18.6%. Instructors with 11 or more years of teaching experience used *authoritative, non-interactive*

discourse a mean of 48.9% with a range of 29.0-80.0%, *authoritative, interactive* a mean of 39.1% with a range of 13.5-57.1%, and *dialogic, interactive* a mean of 8.3% with a range of 0.0-24.4% (Figure 14B, Tables S34, and S35).

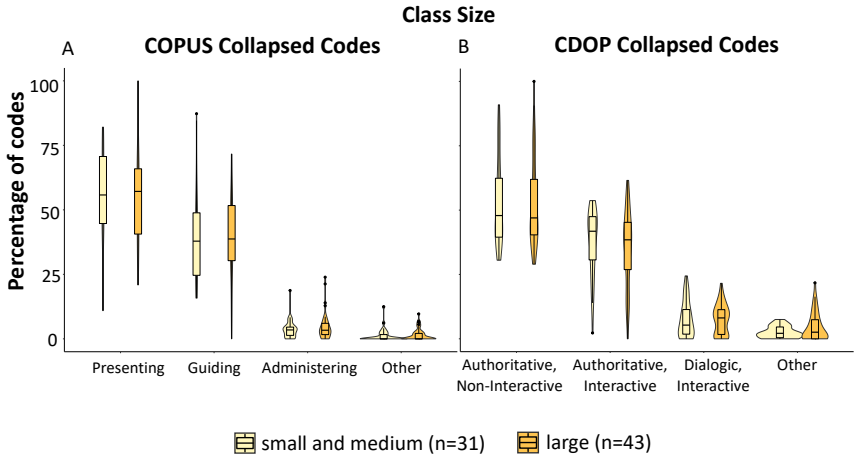
Figure 14. Violin and box-and-whisker plots showing the percentage of codes by instructors' years of teaching experience for instructional practices (A) and discourse practices (B). The violin represents the density of the code frequency. The boxes represent the interquartile range (IQR) of practices for each collapsed code, whiskers represent the largest and smallest values within 1.5 times the IQR, lines within each box represent the median, and the black dot represents the outliers.



Instructional (COPUS) and discourse (CDOP) practices across class size

We did not find significant difference between class size and collapsed COPUS codes on the percentage of codes ($F(3, 288) = 0.11$, $p = 0.95$, $\eta p^2 < 0.001$) (Figure 15A, Tables S36 and S37). Similarly, we did not find significant difference between class size and collapsed CDOP codes on the percentage of codes ($F(3, 288) = 0.43$, $p = 0.73$, $\eta p^2 < 0.001$) (Figure 15B, Tables S38 and S39). We interpret this to mean that there were no significant differences between class size and instructional or discourse practices.

Figure 15. Violin and box-and-whisker plots showing the percentage of codes used by instructors with respect to instructional practices (A) and discourse practices (B) in varying class sizes. The violin represents the density of the code frequency. The boxes represent the interquartile range (IQR) of practices for each collapsed code, whiskers represent the largest and smallest values within 1.5 times the IQR, lines within each box represent the median, and the black dot represents the outliers.



Discussion

A previous study by Kranzfelder et al. (2020) performed on biology instructors teaching in mostly introductory undergraduate biology classes at a research-intensive, predominantly white institution showed that biology instructors *guide* students in active learning activities but still used *authoritative* discourse approaches. In this study, we expanded our understanding of teaching and discourse practices across a range of instructor and course characteristics, including different disciplines instructor types, years of faculty teaching experience, and class size, at a research-intensive HSI. Generally, we found similar general trends in teaching and discourse practices, and that chemistry instructors used mainly teacher-centered instructional and discourse practices, such as *presenting* information to students and *authoritative, non-interactive* discourse practices. Moreover, we found that teaching faculty used more student-centered approaches, such as *guiding* with *dialogic, interactive* practices, than lecturers.

Presenting is associated with authoritative, non-interactive discourse while guiding is associated with dialogic, interactive discourse

We found that *presenting* and *authoritative, non-interactive* were positively correlated to each other while *guiding* was positively correlated to both *interactive* discourse practices. This indicates that when STEM instructors use teacher-centered pedagogies, like lecturing or showing a video, they are most likely the only voice being heard in the classroom (i.e., authoritative). For example, when an instructor is *presenting* content material by mainly lecturing, they dominate the conversations and discuss only their point of view, thus employing the *authoritative, non-*

interactive approach. This magnifies the issue of inclusion in our STEM classrooms, as students traditionally underrepresented in the sciences may not voice their misconceptions or questions when an instructor dominates the conversation. In contrast, students of privileged ethnicities tend to voice their misconceptions and questions regardless of an instructors' teaching style [168]. Additionally, Myers and Rocca (2000) discuss how a "dominant and contentious" communication style leaves students with a negative impression and can adversely impact the student experiences. Conversely, when an instructor is *guiding* students in mainly active learning activities, then they are most likely providing opportunities for the students' point of view and voice to be heard in the classroom and creating opportunities for students to develop their content ideas (i.e., using dialogic, interactive discourse practices). A study done in college classrooms showed that although students' perceptions and peer dynamics influence their participation, instructors play a key role in allowing such participation and student discussions by either controlling the activities and conversations (similar to *presenting* in an *authoritative* manner) or involving students in the learning process (similar to *guiding* with *dialogic* discourse) [70]. Therefore, promoting both student-centered teaching practices (i.e., *guiding*) and student-centered discourse practices (i.e., *dialogic, interactive*) allows for more student involvement and creates an equitable and inclusive learning environment that serves *all* students.

Instructors used mostly presenting and authoritative, non-interactive practices in their college STEM classes

We found that instructors across all STEM disciplines, teaching both lower and upper-division undergraduate and graduate classes, primarily used teacher-centered teaching practices, such as *presenting* information to students and using *authoritative, non-interactive* discourse practices. This trend is prevalent despite evidence of these practices hindering student learning. Prior studies suggest that implementing student-centered, evidence-based teaching practices continues to remain low [170] and college STEM classes are still largely being taught using traditional lecturing, not active learning [95]. Our findings were also consistent with previous studies showing that the teacher-centered discourse patterns were the most prevalent in K-12 classrooms [148,149] and college biology classrooms [97]. Student-centered pedagogies, like *guiding* instructional practices and *dialogic, interactive* discourse practices, could narrow the achievement gap for underrepresented students in STEM fields [171,172]. Also, active learning instructional practices [173] and engaging student discourse promote student learning [140,150,151,173].

To address the question of why STEM instructors continue to use instructor-centered practices, Bathgate et al. (2019) suggest that faculty's perceived barriers, such as time constraints for revising curriculum, requirements for class content, student resistance to active learning, and departmental emphasis on research, could be some of the potential reasons for why we continue to see faculty implementing teacher-centered pedagogies. Additionally, it may be that despite an instructor's plan for active learning activities, they might not be aware of how they are implementing these activities or how they are talking to their students about the content, and thus may continue to dominate the activities and conversations.

Presenting and authoritative, non-interactive dominated instructional practices and TDMs across STEM disciplines

Instructors' guided student engagement can foster student learning with a deeper understanding of scientific ideas [144–146], and classroom observations can help us understand how instructors are implementing these active engagement practices [152]. Prior studies have investigated STEM instructional practices across different instructor and course characteristics, such as STEM discipline, course level, class size, classroom physical layout, and faculty teaching experience [94–96]. Previously, discourse practices were only investigated on biology instructors teaching in mostly introductory undergraduate biology classes at a predominantly white institution [97]. Here we investigated both instructional and discourse practices used across instructors and how these instructional practices correlate with discourse practices across many instructors and class variables.

We found differences in instructional and discourse practices across disciplines, including biology, chemistry, mathematics, and other STEM, similar to other studies [173–175]. When we analyzed the instructional and discourse practices across biology, chemistry, mathematics, and other STEM disciplines, we found that chemistry instructors *presented* more than most STEM discipline instructors and mainly employed *authoritative, non-interactive* discourse. On the other hand, biology instructors used mainly *authoritative* approaches while *guiding* students, comparable to Kranzfelder et al. (2020). Although *dialogic, interactive* discourse was relatively low across all disciplines, other STEM disciplines (physics and engineering) instructors used significantly more *dialogic, interactive* discourse than chemistry instructors, but we did not find other significant differences across the rest of the STEM disciplines. Our findings are supported by recent studies showing that chemistry instructors used little student-centered, collaborative learning pedagogies compared to biology and physics instructors [94,95]. Additionally, our findings are supported by studies from secondary schools where chemistry instructors focus more on knowledge of content material and student misconception and less on instructional delivery and discourse [174,176,177]. This may be due to chemistry instructors employing the same teaching techniques they received while they were students [178]. Conversely, we see that biology instructors used *guiding* instructional practices more than the rest of STEM instructors, and used *dialogic, interactive* discourse practices more than chemistry instructors. Previous literature supports our findings [179–181], as it suggests that biology instructors are frequent implementers of student-centered, active learning strategies. Also, Lund et al. (2015) found that biology instructors used more collaborative learning than other STEM disciplines, and Stains et al. (2018) found that biology instructors used more student-centered instructional practices than other STEM disciplines. It has been shown that inquiry-based learning and team-based learning implemented in biology classrooms promotes student scientific investigation with feedback-rich learning environments [174] and cooperative learning promotes higher concept understanding and information retention [182], so these differences across STEM disciplines could have long-term impacts on student learning.

Teaching faculty used guiding and dialogic, interactive discourse more than lecturers

We found that class sessions taught by teaching faculty used *guiding* teaching practices more than lecturers; moreover, teaching faculty utilized *authoritative*, *interactive* discourse more than lecturers and research faculty. Although we did not see significant differences between lecturers and research faculty with *dialogic, interactive* discourse, we found that teaching faculty used *authoritative, interactive* significantly more than research faculty and lecturers. Although *authoritative, non-interactive,* and *authoritative, interactive* are considered teacher-centered discourse practices [90], *authoritative, interactive* allows for more student involvement than *authoritative, non-interactive* approaches. These findings are not surprising based on the roles and expectations of the three studied instructor types. Xu and Solanki (2020) describe teaching faculty as tending to have more teaching professional development opportunities than research faculty and lighter teaching loads when compared to lecturers. Xu & Solanki (2020) also found that students who take their initial course with teaching faculty do slightly better in subsequent courses than those who take these courses with lecturers or research faculty. Additionally, teaching and teaching-related professional development certifications are not weighted heavily for tenure and promotion purposes; thus, research faculty may lack both the time and motivation needed to improve their instructional quality [161]. Lecturers have expectations to teach heavier course loads with little consistency of courses taught from one term to the next [98]. Additionally, lecturers have relatively low compensation, minimal benefits, limited participation in departmental decisions, and lack of job security, leading to a lack of motivation for professional development to improve their teaching skills [98,183,184]. Taken together, we conclude that teaching and research faculty *guide* their students through active learning activities, and teaching faculty tend to involve students more in the conversations, especially using *authoritative, interactive* discourse.

Years of faculty teaching experience does not have a significant impact on instructional or discourse practices

We found that instructors' years of faculty teaching experiences did not impact instructional or discourse practices. This was somewhat surprising to us as these STEM instructors had a spectrum of years of faculty teaching experience at this institution (0-11+ years). We expected instructors with more years of faculty teaching experience to implement more student-centered classroom practices. For example, Berger et al. (2018) found that instructors with more teaching experience increased opportunities for student involvement. In addition, they found that instructors with more experience gained a better understanding of classroom management, and therefore, improved communication with their students [167]. Additionally, Keavney and Sinclair (1978) stated that novice instructors have teaching anxiety that diminishes with teaching experience. Furthermore, Bathgate et al. (2019) found that as instructors gain teaching experience, they become confident in their practices, and tend to engage in more discursive, open-ended classroom discussions. Also, Hoy and Spero (2005) stated that the efficacy of teaching practices might be low in earlier years of teaching but increase with experience. Although most literature describes how teaching practices improve and marches towards more student-centered practices with more experience, we cannot discern that with our results. A possible explanation to why our results do not reflect what has been observed in other studies could be due to lack of buy-in [186], professional identity of the instructors [159], and/or perceived student resistance to active learning strategies [187].

Moreover, other studies have found other resource and time barriers to implementing active learning, such as lack of time for preparations of class material and in-class active learning activities, lack of technology that supports in-class active learning, lack of training, lack of incentives, and lack of administrative support [186,188,189]. Implementing active learning in STEM classrooms requires buy-in, resources, and time from instructors, students, and administration; thus, if instructors are not sold on the benefits of active learning and lack such resources, then they are less likely to implement active learning regardless of how long they have taught at the institution. Despite the lack of significant differences, instructors in our study had a wide range of years of teaching experience within each category, but they are all predisposed to their own beliefs, knowledge and skills. For example, two faculty with 6 years of teaching experience might have different pedagogical beliefs and knowledge, and therefore, may implement active learning to varying degrees.

Class size did not affect instructional and discourse practices

Although it has been reported by faculty that large class size hinders the implementation of active learning due to the physical structure of the room, inability to hear students' responses, and the dominance of responses from a small percentage of students [190,191], we found that neither instructional practices nor the discourse practices differed across class sizes. Smith et al. (2014) and our study did not find differences across class sizes. Furthermore, Akiha et al. (2018) reported that even in small class sizes (30 and below), instructors continue to *present*, indicating that class size did not affect instructional practices of the instructors in their study context. However, in support of our findings, Lund et al. (2015) showed that large enrollment classes were not a barrier to implementing student-centered instruction, and Stains et al. (2018) found that small course sizes did not necessarily lead to the implementation of more student-centered instructional practices. Therefore, it should be reassuring to STEM instructors of all class size to find that active learning could be implemented in STEM classrooms irrespective of the class size.

Methods and Materials

Institution and instructor population

We compared 35 instructors teaching 74 in-person class sessions in undergraduate and graduate STEM courses, including biology, chemistry, mathematics, physics, and engineering, at a mid-sized, public, research-intensive university designated as an HSI. Table 13 shows the characteristics of the instructors and their courses. The instructor type varied between tenure-track/tenured research faculty (referred to as "research faculty" hereafter), tenure-track/tenured teaching faculty (referred to as "teaching faculty" hereafter), and non-tenure-track contingent faculty (referred to as "lecturers" hereafter). The years of teaching experience is based on the number of years teaching as the instructor of record (IOR) at this institution. Years of teaching experience ranged from 0 to 10 or more years, with 40% of all instructors having more than 10

years of teaching experience. Participating instructors varied across STEM departments, with the majority being in biology, followed by chemistry, mathematics, and other STEM (engineering and physics). In addition, courses were mostly taught by a sole instructor (i.e., not co- or team-teaching), and the class sizes ranged from 4 to 292 students, with the mean class size being 110 students. Our instructors taught mainly lower-division courses that were designated for majors (Table 13).

Characteristics	*n*	%
Years of teaching experience		
0-5	14	40.0
6-10	8	22.9
11+	13	37.1
Instructor type		
Research faculty	14	40.0
Teaching faculty	7	20.0
Lecturers	14	40.0
STEM discipline of instructor		
Biology	16	45.7
Molecular and Cellular Biology	(12)	
Life and Environmental Sciences	(2)	
Quantitative Systems Biology	(2)	
Chemistry	9	25.7
Mathematics	4	11.4
Other STEM	6	27.1
Physics	(4)	
Engineering	(2)	
Class size (class sessions)		
Small (≤60 students)	24	32.4
Medium (61-100 students)	6	8.1
Large (>100 students)	43	59.5
Class level (class sessions)		
Lower division	55	74.3
Upper division	14	18.9
Graduate	5	6.8

Table 13. Demographic characteristics of instructors (n = 35) and their courses (74 class sessions). Note: Some instructors taught more than one course, but demographics and class sessions are included per instructor. Parentheses indicate numbers in the subcategory.

Instructor Recruitment

We sent out an initial recruitment email to research and teaching faculty through faculty department email list serves and individual emails to lecturers in the departments of biology, chemistry, physics, and mathematics. Also, we sent out individual emails to teaching faculty in engineering. This initial email included the purpose of our study, procedures, benefits, IRB approval, potential dissemination of results, classroom observation scheduling information, and contact information for questions. We invited instructors to participate in our study that met the following selection criteria: 1) taught either an undergraduate or graduate STEM course, 2) taught the lecture component of the course, not laboratory or discussion, and 3) taught the course in-person between two academic years (Fall 2018, Spring 2019, Fall 2019, and Spring 2020 semesters (pre-COVID-19 global pandemic)). Initially, 41 instructors consented to participate in the study;

however, two were excluded due to classroom observation scheduling conflicts, two were excluded due to either being a lab or discussion component of the course, and two were excluded as they did not teach in-person after the transition to emergency remote instruction during the COVID-19 global pandemic in the Spring 2020 semester. We are unable to give the participation rate as the total number of instructors in the email listserves is unknown. The study was classified by the UC Merced Institutional Review Board as exempt (Protocol ID UCM2020-3).

Classroom Observation Recordings

We collected audio recordings from one to three class sessions for each of the instructors using either a Sony HDR camcorder with a microphone or a Swivl™ with a remote marker and an Apple iPad. Class sessions ranged from 38 to 82 minutes, avoiding class sessions where the entire meeting time was dedicated to exams, student presentations, or special group project work. However, we included class sessions in which quizzes were given since these are a regular part of the daily or weekly class sessions and only took 10-20 minutes.

COPUS Data Collection

We used COPUS to quantify the instructional practices observed across instructors and compared them across STEM disciplines, instructor types, years of faculty teaching experience, and class size. We collected live classroom observational data using the Classroom Observation Protocol for Undergraduate STEM (COPUS), which provides reliable data by documenting the co-occurrence of 12 instructor behaviors (e.g., *lecturing*) and 13 student behaviors (e.g., *listening*) during two-minute time intervals over the entire class session [88] (Tables S11 and S12). We followed the code description outlined by Smith et al. 2013, with the exception that *one-on-one discussions* were coded by observers when the instructor was helping one student or a small group and not paying attention to the rest of the class and *whole-class discussion* was coded when students were leading a discussion, such as an in-class debate or Socratic seminar. We combined the 25 individual codes into four collapsed instructor and student codes, adapted from Smith et al. (2014) and categorized by Kranzfelder et al. (2019). For instructors, the collapsed codes were: 1) Presenting, 2) Guiding, 3) Administering, and 4) Other. For this study, we only looked at instructor codes.

The live COPUS observations were conducted by 14 undergraduate student interns working for a branch of the Center for Engaged Teaching and Learning, called Students Assessing Teaching And Learning (SATAL), at the institution of study. SATAL interns support faculty and staff's professional development by observing their teaching and learning through COPUS observations, class interviews, and focus groups and provide instructors with actionable feedback. Before collecting observation data, SATAL interns were trained to conduct COPUS in 3 hours by three of the authors (JA, AMS, and PK) according to the training outlined in until substantial inter-rater reliability (IRR) was established between all coders ($k = 0.55$, 95% CI: 0.54-0.56) (Table S15). In addition to having a substantial IRR, student interns met for up to 30-minutes after each classroom observation to discuss their codes and resolve any coding disagreements until reaching 100% consensus. By having both a substantial kappa score and consensus building after the classroom observation, the data collected by student interns were considered reliable. At minimum, two SATAL interns were present in the classroom for each of the live observations.

CDOP Data Collection

We used CDOP to quantify the discourse practices observed across instructors and compared them across STEM disciplines, instructor types, years of faculty teaching experience, and class size. We listened to audio recordings while using the Classroom Discourse Observation Protocol (CDOP) to quantify the TDMs used by our instructors [90] (Tables S13 and S14). We used the CDOP to document TDMs used by our instructors in two-minute time intervals over the class session, with 15 content-related codes, 1 non-content code, and 1 code for any new discourse that is not represented by any of the given codes. The codes are collapsed into four discourse practices: (1) Authoritative, Non-Interactive (e.g., sharing information), (2) Authoritative, Interactive (e.g., asking generative questions), (3) Dialogic, Interactive (e.g., asking students to challenge each other's work), (4) and Other (e.g., discussing class logistics). One coder (JA) was trained for 3 hours by the corresponding author (PK), while two coders (CD & AHS) were trained by the first author (JA) according to the training outlined in until substantial IRR was established between all four coders (k: 0.79, CI 0.72-0.86, Table S16). Over several months, three coders (JA, CD, & AHS) independently coded as first coders for 74 audio recordings using the CDOP, while two coders (PK & JA) served as second coders for 20 of the audio recordings (i.e., 27%, k = 0.83, Table S17). If the average Kappa score for independent coding was not substantial (i.e., below 0.6), we discussed coding discrepancies until reaching consensus between coders.

Data Analyses

Following Kranzfelder et al. (2020), Lewin et al. (2016), and Meaders et al. (2019), we analyzed the COPUS and CDOP individual codes using the percentage of two-min time intervals to determine and compare the frequency of a particular code. In particular, we divided the number of two-min time intervals marked for each code (e.g., *sharing*) by the total number of two-min time intervals for that class session. For example, if *sharing* was marked 20 of the two-min time intervals out of a possible 30 two-min time intervals (i.e., 60-min class session), then 20/30 or 66.7% of the possible two-min time intervals contained *sharing*. This calculation slightly overestimates the amount of time an instructor spends on any one behavior as the behavior is counted for the entire two-min time interval even if the instructor only spends 10 seconds on it.

Similar to, Smith et al. (2014), Kranzfelder et al. (2020), and Lewin et al. (2016), we analyzed the COPUS and CDOP collapsed data using the percentage of codes to get a more holistic view of multiple codes and compare across broad instructional practices. Also, we analyzed COPUS and CDOP collapsed data by the percentage of codes to determine differences across STEM disciplines, instructor types, years of faculty teaching experience, and class sizes. More specifically, we added the total number of times each code was marked and divided it by the total number of codes. For example, if *sharing* was marked 20 times and there were 50 codes in total, then *sharing* would correspond to 20/50 or 40% of the total codes. This calculation slightly underestimates the amount of time an instructor spends on any one behavior as it counts the behavior relative to all other behaviors.

We categorized our data to quantify how instructional and discourse practices differed between (1) instructors' STEM disciplines, (2) instructor types, (3) years of teaching experiences,

and (4) class sizes. First, we divided the STEM disciplines into four categories: (a) biology (molecular and cellular biology, quantitative and systems biology, life and environmental sciences), (b) chemistry, (c) mathematics, and (d) other STEM (engineering and physics). Second, we divided instructor types into three categories following categorization from: (a) research faculty, (b) teaching faculty, and (c) lecturers. Third, we divided the years of faculty teaching experience based on the number of years teaching as the instructor of record at this institution of study into three categories: (a) 0-5 years, (b) 6-10 years, and (c) >10 years. Fourth, we divided the class size (or the number of students per class) into two categories: (a) small (≤60 students) and medium (61-100), and (b) large (>100 students). We made categories that were based on samples with at least 10 class sessions for all four variables.

Statistical Analyses

We used non-parametric Spearman's Rank Correlation tests to determine if there were relationships between instructional and discourse practices across instructors. More specifically, we correlated two COPUS collapsed instructor codes (*presenting* and *guiding*) to three CDOP collapsed codes (*authoritative, non-interactive*; *authoritative, interactive*; and *dialogic, interactive*). We explored the relationships of *presenting* and *guiding* to the three discourse approaches as these instructional practices create opportunities for conversations between instructors and students around content. We used non-parametric Friedman tests and pairwise comparisons using Wilcoxon signed-rank tests with Bonferroni corrections to calculate significant differences in the medians of COPUS collapsed codes (i.e., general instructional practices) and CDOP collapsed codes (i.e., general discourse practices) across instructors. In addition, we used Kendall's W test (W) for calculating effect size, which uses the Cohen's interpretation guidelines of 0.1 to 0.3 (small effect), 0.3 to 0.5 (moderate effect), and greater than 0.5 (large effect) [194,195].

To determine if there were differences between instructional and discourse practices by STEM discipline of the course, instructor type, years of teaching experience, and class size, we conducted a non-parametric Aligned Ranks Transformation ANOVA [196] with the *ARTool* package in R [197]. In addition, we used the partial eta-squared measure (ηp^2) for calculating effect size, which uses 0.01 to 0.06 to indicate a small effect, 0.06 to 0.14 to indicate a moderate effect, and greater than 0.14 to indicate a large effect [194,195]. All statistical analyses were conducted using the R statistical software [198] and the significance threshold (p value) was set at 0.05 for all tests.

Chapter 5: Conclusion

Antibiotic Resistance

Antibiotic resistant infections have become a global crisis, claiming the lives of nearly 700,000 people yearly. Microbes have the ability to share resistance genetic material via many mechanisms, which exacerbates the problem and renders many once life-saving antibiotics ineffective [14,66]. Together with dwindling discovery and development of new antibiotic [3], it is of vital importance to study patterns and causes of antibiotic resistance to ensure the prolonged activities of the remaining effective antimicrobial therapies.

Ceftolozane/tazobactam (c/t)

Ceftolozane/tazobactam (c/t) is a relatively new antibiotic/inhibitor combination drug that shows promising results treating clinical infections that were otherwise resistant [199] and displays low *in vitro* resistance rates [56–58]. To monitor the continued antimicrobial ability of this promising new antimicrobial therapy, we assessed the local patterns of c/t resistance in Extended Spectrum β-lactamase (ESBL)-producing isolates collected mainly from urinary tract infections (UTIs) at Dignity Health Mercy Medical Center (DHMMC), an agricultural-serving community hospital. Furthermore, investigated mechanisms of resistance by associating candidate resistance genes with the zone of inhibition (ZI) measurements using the Kirby-Bauer disk diffusions tests. Since c/t is a combination therapy for UTI treatment that is insensitive to many ESBLs [55,128,199], and since our isolates are ESBL+ and mainly collected from patients with UTIs, we have a good setup to investigate the patterns of resistance and what predict the likelihood of its continued efficacy.

The Study

Our surveillance study showed a low c/t resistance rate which is consistent with other national and global c/t surveillance studies [56–58]. We also looked at associations between c/t ZI measurements and the four common ESBL genes *bla*$_{\text{TEM}}$, *bla*$_{\text{OXA}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{CTX-M}}$. Our data showed that expression of *bla*$_{\text{CTX-M's}}$ and *bla*$_{\text{SHV's}}$ were independently associated with smaller ZI, thus they are contributing to c/t resistance. In our collection, we also found many isolates that contained either *bla*$_{\text{SHV}}$, or *bla*$_{\text{CTX-M}}$, indicating that their sole presence is not sufficient to drive resistance; rather, a combination between these genes and non-ESBL genes is necessary to achieve resistance. To determine the presence of potential non-ESBL genes, we examined genomic sequence data for ESBL isolates and found that OmpK35 and OmpK37 were associated with c/t resistance, and that the presence of *emrD* with each of *ramR*, *ompK35*, and *ompK37* was also associated with c/t resistance. To confirm our findings from the genomic data, we PCR screened a subset of the tested isolates for the presence of *emrD*, *ompK35*, *ompK37*, and *ramR*, and found that we lost all significant associations, meaning that not one of these genes, nor a combination of them, significantly contributed to a elevated c/t resistance.

We investigated if resistance to c/t was associated with co-expression of ESBL and non-ESBL genes, again, we did not find any significant associations, especially after running the False Discovery Rate controlling procedure. Although literature confirms the presence of each CTX-M, SHV, and the *absence* of OmpK35 has been found to contribute to c/t resistance [46,58,61,128], our results only confirm the association of the two β-lactamases (*bla*$_{\text{CTX-M}}$ and *bla*$_{\text{SHV}}$) with c/t resistance.

Overall, we sought to contribute to literature by performing multiple investigations. Our findings support literature on many fronts, especially that c/t has a lower rate of resistance and that two ESBL genes (*bla*$_{\text{CTX-M}}$ and *bla*$_{\text{SHV}}$) contribute to resistance, while it is insensitive to non-ESBL genes. We added to existing literature that c/t has a complex resistance phenotype where not any single gene is capable of conferring resistance on its own.

Study Limitations

The surveillance sample used in this study was large (n = 993) and comparable to some other surveillance studies (REF). Although it included a large sample size for the ESBL PCR screening (n = 852), the sample size for genomic sequences (n = 123) and non-ESBLs (n = 96) was small. Having a larger data set would have made our results more robust and may have produced different outcomes. For the non-ESBL PCR screen, we decided to have a uniform set of isolates from each represented category, 32 resistant, 32 intermediate, 32 susceptible, thus limiting the total number of PCR screen isolates to 96. The possibility of finding more connections between non-ESBLs and c/t resistance or between non-ESBLs, ESBLs and c/t resistance could have been prominent had the sample size been larger.

Future Directions

Future work could be directed towards conducting more Kirby-Bauer disk diffusion assays on isolates collected after the surveillance was performed to see continue monitoring resistance trends. Additionally, expanding the PCR screen for non-ESBLs on a larger subset of the entire collection would increase the robustness of the results.

Education Research

Conducting discipline-based education research (DBER) to examine instructional and discourse practices in STEM classrooms is vital to understand patterns of teaching. The importance of these investigations stems from the need to improve college STEM education by improving communications of scientific information and emphasizing student-centered teaching practices. As a first step, we studied the patterns of instructional and discourse practices that are currently taking place in college STEM courses at an Hispanic Serving Institution (SHI). We studied instructors (n = 35) teaching the lecture component of undergraduate (n = 32) and graduate (n = 3) STEM courses across instructor

and course variables, such as discipline, appointment line, years of faculty teaching experience, and class size. We found that out of all the disciplines studied, chemistry instructors used more instructor-centered instructional and discourse practices than biology instructors. Additionally, we found that teaching faculty used more student-centered teaching approaches than lecturers, but not research faculty. We did not find associations between either the years of faculty teaching experience nor class size on the instructor's teaching practices (instructional and discourse).

Through this DBER study, we were able to find the patterns of instructional and discourse practices enacted by STEM instructors. Our findings will help instructors reflect on their teaching practices and consider if they are planning active learning activities in the classroom and if they are using *dialogic interactive* discourse. We found that although some instructors use active learning instructional practices, they are using *authoritative* discourse; students are involved in class activities, but in the conversations, they are getting to do activities, but not discuss them. These patterns are very informative and can help university departments provide targeted faculty teaching professional development (TPD) opportunities. TPD could be in the form of workshops or seminars that help instructors implement more active learning and dialogic discourse. These workshops and/or seminar give instructors recommendations such as employing more think-pair-share opportunities for students, implementing small group discussions, and asking students to come up with conclusions and having them give reasons to their answers. It is important that institutions provide opportunities for TPD to their faculty, so that students get the maximum benefit of learning in their STEM classes.

Limitations and Future Study

There are limitations to our study that could be addressed in future studies. For example, although there is evidence of improved student performance with student-centered teaching approaches [77,79,84,85], we did not collect student performance data to assess learning and study its relationship with the instructional and discourse patterns observed. Additionally, a future study could be to investigate instructional and discourse practices across multiple institutions, especially Minority Serving Institutions (MSI) such as UC Merced, and with the variables studied here. Such research would investigate if the patterns observed here resemble those of other MSI institutions, and how that compares to investigations performed at Primarily Whites Institutions. Results from such studies could be generalizable and may hold true beyond the classroom and into communities of different socioeconomical and ethnic backgrounds.

In conclusion, I am proud to have interdisciplinary research training that combines microbial evolution and antibiotic resistance bench work with quantitative education research. To my knowledge, I am the first graduate student to have such interdisciplinary thesis, combining two distinct disciplines into one dissertation. Breaking the boundaries between disciplines can contribute to the broader dissemination of knowledge and allows

those involved to apply their acquired skills in multiple settings. In the future, I hope to continue down the same path of interdisciplinary work and diminish distances between discoveries in scientific communities and non-scientific communities, joining forces to build stronger societies with informed citizens.

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Appendix

Table of Contents

Chapter 2 supplementals

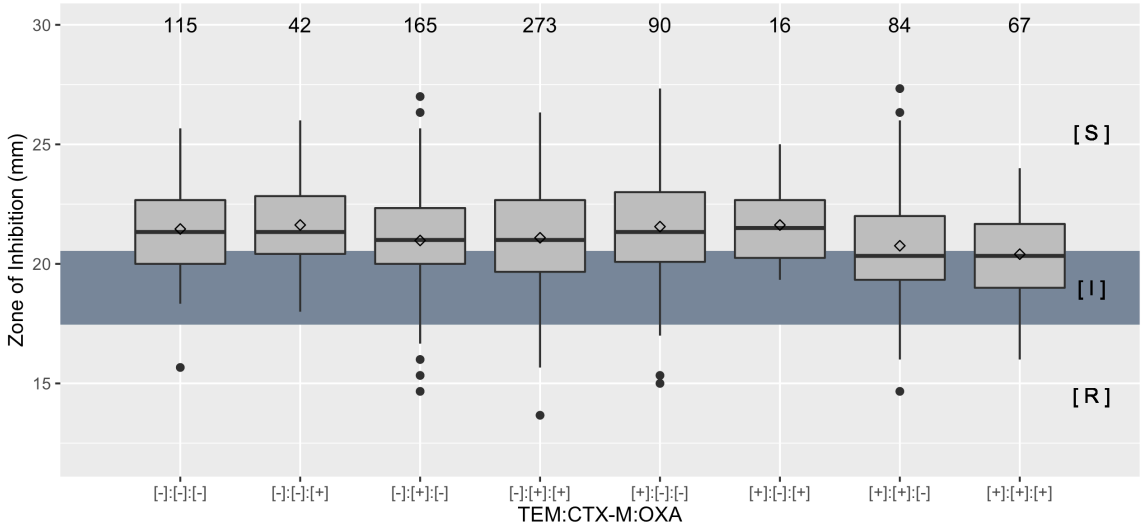

Figure S1. (*bla*$_{TEM}$: *bla*$_{CTX-M}$: *bla*$_{OXA}$). Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by $[+]$ and resistance gene absence is indicated by $[-]$. The diamond $(\diamond)$ indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible $([\mathbf{S}])$, intermediate $([\mathbf{I}])$, and Resistant $([\mathbf{R}])$.

bla_{TEM}: $bla_{CTX\text{-}M}$: bla_{OXA}	Difference	p-value	95% CI
$\mu([-]:[-]:[-]) - \mu([-]:[-]:[+])$	-0.168	0.99980	(-1.303,0.968)
$\mu([-]:[-]:[-]) - \mu([-]:[+]:[-])$	0.481	0.54770	(-0.284,1.246)
$\mu([-]:[-]:[-]) - \mu([-]:[+]:[+])$	0.368	0.75500	(-0.332,1.068)
$\mu([-]:[-]:[-]) - \mu([+]:[-]:[-])$	-0.100	1.00000	(-0.986,0.786)
$\mu([-]:[-]:[-]) - \mu([+]:[-]:[+])$	-0.166	1.00000	(-1.846,1.515)
$\mu([-]:[-]:[-]) - \mu([+]:[+]:[-])$	0.701	0.26520	(-0.202,1.605)
$\mu([-]:[-]:[-]) - \mu([+]:[+]:[+])$	1.054	0.02162	(0.086,2.022)
$\mu([-]:[-]:[+]) - \mu([-]:[+]:[-])$	0.648	0.61650	(-0.440,1.736)
$\mu([-]:[-]:[+]) - \mu([-]:[+]:[+])$	0.535	0.77720	(-0.508,1.579)
$\mu([-]:[-]:[+]) - \mu([+]:[-]:[-])$	0.068	1.00000	(-1.109,1.244)
$\mu([-]:[-]:[+]) - \mu([+]:[-]:[+])$	0.002	1.00000	(-1.848,1.852)
$\mu([-]:[-]:[+]) - \mu([+]:[+]:[-])$	0.869	0.34340	(-0.321,2.059)
$\mu([-]:[-]:[+]) - \mu([+]:[+]:[+])$	1.222	0.05672	(-0.018,2.461)
$\mu([-]:[+]:[-]) - \mu([-]:[+]:[+])$	-0.113	0.99940	(-0.734,0.508)
$\mu([-]:[+]:[-]) - \mu([+]:[-]:[-])$	-0.580	0.39400	(-1.406,0.245)
$\mu([-]:[+]:[-]) - \mu([+]:[-]:[+])$	-0.646	0.93560	(-2.295,1.003)
$\mu([-]:[+]:[-]) - \mu([+]:[+]:[-])$	0.221	0.99350	(-0.623,1.065)
$\mu([-]:[+]:[-]) - \mu([+]:[+]:[+])$	0.573	0.54740	(-0.339,1.485)
$\mu([-]:[+]:[+]) - \mu([+]:[-]:[-])$	-0.468	0.58420	(-1.233,0.298)
$\mu([-]:[+]:[+]) - \mu([+]:[-]:[+])$	-0.533	0.97480	(-2.153,1.086)
$\mu([-]:[+]:[+]) - \mu([+]:[+]:[-])$	0.334	0.90390	(-0.452,1.119)
$\mu([-]:[+]:[+]) - \mu([+]:[+]:[+])$	0.686	0.23040	(-0.172,1.545)
$\mu([+]:[-]:[-]) - \mu([+]:[-]:[+])$	-0.066	1.00000	(-1.774,1.643)
$\mu([+]:[-]:[-]) - \mu([+]:[+]:[-])$	0.801	0.17770	(-0.154,1.757)
$\mu([+]:[-]:[-]) - \mu([+]:[+]:[+])$	1.154	0.01345	(0.138,2.170)
$\mu([+]:[-]:[+]) - \mu([+]:[+]:[-])$	0.867	0.79130	(-0.851,2.585)
$\mu([+]:[-]:[+]) - \mu([+]:[+]:[+])$	1.220	0.40850	(-0.533,2.972)
$\mu([+]:[+]:[-]) - \mu([+]:[+]:[+])$	0.352	0.96910	(-0.679,1.384)

Table S2. (bla_{TEM}: $bla_{CTX\text{-}M}$: bla_{OXA}): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by bla_{TEM} $bla_{CTX\text{-}M}$, and bla_{OXA} Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value. p-value<0.05 is significant.

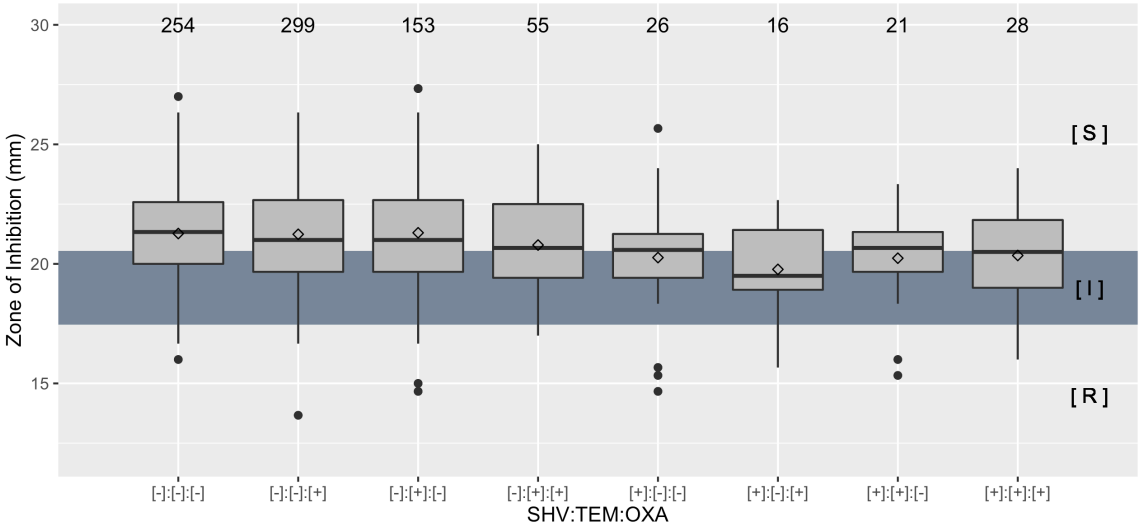

Figure S2. (*bla*$_{SHV}$: *bla*$_{TEM}$: *bla*$_{OXA}$): Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by $[+]$ and resistance gene absence is indicated by $[-]$. The diamond $(\diamond)$ indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible $([\mathbf{S}])$, intermediate $([\mathbf{I}])$, and Resistant $([\mathbf{R}])$.

bla_{SHV}: bla_{TEM}: bla_{OXA}	Difference	p-value	95% CI
$\mu([-]:[-]:[-]) - \mu([-]:[-]:[+])$	0.032	1.00000	(-0.505,0.569)
$\mu([-]:[-]:[-]) - \mu([-]:[+]:[-])$	-0.031	1.00000	(-0.675,0.613)
$\mu([-]:[-]:[-]) - \mu([-]:[+]:[+])$	0.482	0.77370	(-0.454,1.417)
$\mu([-]:[-]:[-]) - \mu([+]:[-]:[-])$	1.007	0.26330	(-0.288,2.302)
$\mu([-]:[-]:[-]) - \mu([+]:[-]:[+])$	1.499	0.09440	(-0.122,3.120)
$\mu([-]:[-]:[-]) - \mu([+]:[+]:[-])$	1.032	0.35820	(-0.397,2.460)
$\mu([-]:[-]:[-]) - \mu([+]:[+]:[+])$	0.918	0.33790	(-0.334,2.171)
$\mu([-]:[-]:[+]) - \mu([-]:[+]:[-])$	-0.063	1.00000	(-0.688,0.562)
$\mu([-]:[-]:[+]) - \mu([-]:[+]:[+])$	0.450	0.82020	(-0.473,1.372)
$\mu([-]:[-]:[+]) - \mu([+]:[-]:[-])$	0.975	0.29510	(-0.311,2.261)
$\mu([-]:[-]:[+]) - \mu([+]:[-]:[+])$	1.467	0.10690	(-0.147,3.081)
$\mu([-]:[-]:[+]) - \mu([+]:[+]:[-])$	0.999	0.39340	(-0.421,2.419)
$\mu([-]:[-]:[+]) - \mu([+]:[+]:[+])$	0.886	0.37580	(-0.357,2.129)
$\mu([-]:[+]:[-]) - \mu([-]:[+]:[+])$	0.513	0.76750	(-0.476,1.502)
$\mu([-]:[+]:[-]) - \mu([+]:[-]:[-])$	1.038	0.26260	(-0.296,2.372)
$\mu([-]:[+]:[-]) - \mu([+]:[-]:[+])$	1.530	0.09353	(-0.123,3.183)
$\mu([-]:[+]:[-]) - \mu([+]:[+]:[-])$	1.063	0.35150	(-0.401,2.526)
$\mu([-]:[+]:[-]) - \mu([+]:[+]:[+])$	0.949	0.33610	(-0.343,2.242)
$\mu([-]:[+]:[+]) - \mu([+]:[-]:[-])$	0.525	0.96430	(-0.972,2.022)
$\mu([-]:[+]:[+]) - \mu([+]:[-]:[+])$	1.017	0.67060	(-0.770,2.804)
$\mu([-]:[+]:[+]) - \mu([+]:[+]:[-])$	0.550	0.96960	(-1.064,2.163)
$\mu([-]:[+]:[+]) - \mu([+]:[+]:[+])$	0.437	0.98560	(-1.024,1.897)
$\mu([+]:[-]:[-]) - \mu([+]:[-]:[+])$	0.492	0.99560	(-1.507,2.491)
$\mu([+]:[-]:[-]) - \mu([+]:[+]:[-])$	0.025	1.00000	(-1.821,1.870)
$\mu([+]:[-]:[-]) - \mu([+]:[+]:[+])$	-0.088	1.00000	(-1.801,1.625)
$\mu([+]:[-]:[+]) - \mu([+]:[+]:[-])$	-0.467	0.99760	(-2.555,1.620)
$\mu([+]:[-]:[+]) - \mu([+]:[+]:[+])$	-0.580	0.98680	(-2.552,1.391)
$\mu([+]:[+]:[-]) - \mu([+]:[+]:[+])$	-0.113	1.00000	(-1.929,1.703)

Table S3. (bla_{SHV}: bla_{TEM}: bla_{OXA}): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by bla_{SHV}, bla_{TEM}, and bla_{OXA} Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value. p-value<0.05 is significant

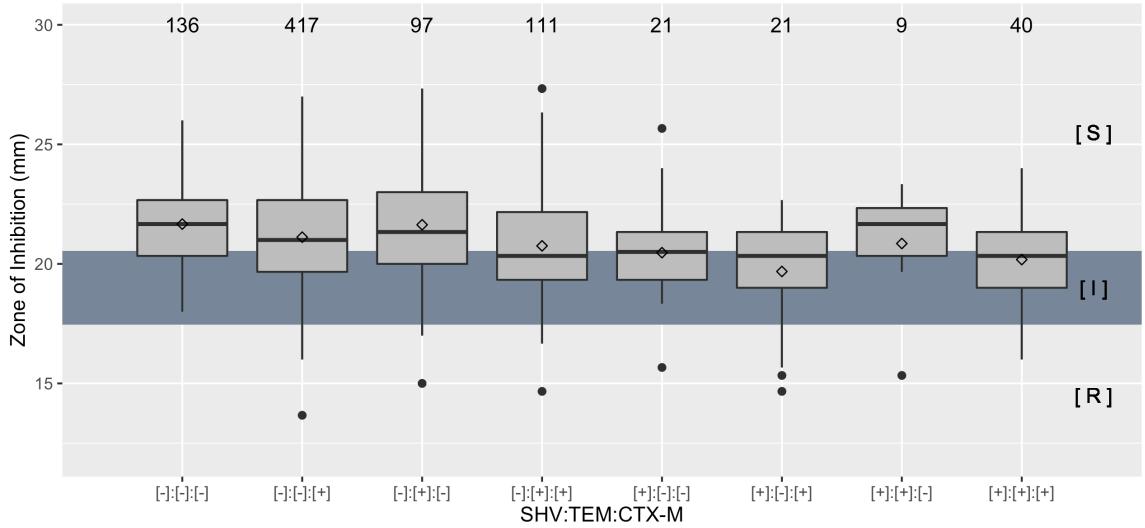

Figure S3. (*bla*$_{SHV}$: *bla*$_{TEM}$: *bla*$_{CTX-M}$): Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by $[+]$ and resistance gene absence is indicated by $[-]$. The diamond $(\diamond)$ indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible $([\mathbf{S}])$, intermediate $([\mathbf{I}])$, and Resistant $([\mathbf{R}])$.

bla_{SHV}: bla_{TEM}: $bla_{CTX\text{-}M}$	Difference	p-value	95% CI
$\mu([-]:[-]:[-]) - \mu([-]:[-]:[+])$	0.546	0.12520	(-0.069,1.162)
$\mu([-]:[-]:[-]) - \mu([-]:[+]:[-])$	0.028	1.00000	(-0.800,0.857)
$\mu([-]:[-]:[-]) - \mu([-]:[+]:[+])$	0.910	0.01255	(0.113,1.708)
$\mu([-]:[-]:[-]) - \mu([+]:[-]:[-])$	1.196	0.20350	(-0.265,2.657)
$\mu([-]:[-]:[-]) - \mu([+]:[-]:[+])$	1.982	0.00103	(0.521,3.443)
$\mu([-]:[-]:[-]) - \mu([+]:[+]:[-])$	0.812	0.94610	(-1.332,2.957)
$\mu([-]:[-]:[-]) - \mu([+]:[+]:[+])$	1.485	0.00153	(0.364,2.606)
$\mu([-]:[-]:[+]) - \mu([-]:[+]:[-])$	-0.518	0.33100	(-1.220,0.185)
$\mu([-]:[-]:[+]) - \mu([-]:[+]:[+])$	0.364	0.71430	(-0.301,1.030)
$\mu([-]:[-]:[+]) - \mu([+]:[-]:[-])$	0.650	0.85170	(-0.744,2.043)
$\mu([-]:[-]:[+]) - \mu([+]:[-]:[+])$	1.435	0.03812	(0.042,2.829)
$\mu([-]:[-]:[+]) - \mu([+]:[+]:[-])$	0.266	0.99990	(-1.833,2.366)
$\mu([-]:[-]:[+]) - \mu([+]:[+]:[+])$	0.939	0.10570	(-0.093,1.970)
$\mu([-]:[+]:[-]) - \mu([-]:[+]:[+])$	0.882	0.04240	(0.016,1.748)
$\mu([-]:[+]:[-]) - \mu([+]:[-]:[-])$	1.167	0.26170	(-0.332,2.667)
$\mu([-]:[+]:[-]) - \mu([+]:[-]:[+])$	1.953	0.00202	(0.453,3.453)
$\mu([-]:[+]:[-]) - \mu([+]:[+]:[-])$	0.784	0.95820	(-1.388,2.955)
$\mu([-]:[+]:[-]) - \mu([+]:[+]:[+])$	1.457	0.00406	(0.286,2.628)
$\mu([-]:[+]:[+]) - \mu([+]:[-]:[-])$	0.285	0.99910	(-1.197,1.768)
$\mu([-]:[+]:[+]) - \mu([+]:[-]:[+])$	1.071	0.35800	(-0.412,2.554)
$\mu([-]:[+]:[+]) - \mu([+]:[+]:[-])$	-0.098	1.00000	(-2.258,2.062)
$\mu([-]:[+]:[+]) - \mu([+]:[+]:[+])$	0.575	0.79940	(-0.575,1.724)
$\mu([+]:[-]:[-]) - \mu([+]:[-]:[+])$	0.786	0.92060	(-1.137,2.709)
$\mu([+]:[-]:[-]) - \mu([+]:[+]:[-])$	-0.384	0.99980	(-2.866,2.099)
$\mu([+]:[-]:[-]) - \mu([+]:[+]:[+])$	0.289	0.99960	(-1.390,1.968)
$\mu([+]:[-]:[+]) - \mu([+]:[+]:[-])$	-1.169	0.84470	(-3.652,1.313)
$\mu([+]:[-]:[+]) - \mu([+]:[+]:[+])$	-0.497	0.98650	(-2.176,1.183)
$\mu([+]:[+]:[-]) - \mu([+]:[+]:[+])$	0.673	0.98730	(-1.626,2.972)

Table S4. (bla_{SHV}: bla_{TEM}: $bla_{CTX\text{-}M}$): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value. p-value<0.05 is significant.

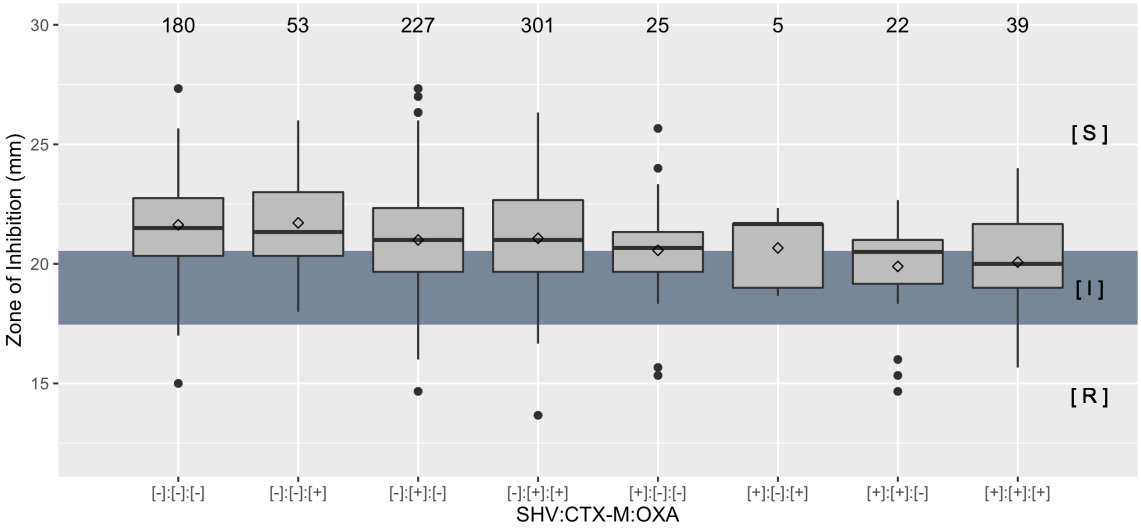

Figure S4. (*bla*$_{SHV}$: *bla*$_{CTX-M}$: *bla*$_{OXA}$): Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by $[+]$ and resistance gene absence is indicated by $[-]$. The diamond $(\diamond)$ indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible $([\mathbf{S}])$, intermediate $([\mathbf{I}])$, and Resistant $([\mathbf{R}])$.

bla_{SHV}: bla_{CTX-M}: bla_{OXA}	Difference	p-value	95% CI
$\mu([-]:[-]:[-]) - \mu([-]:[-]:[+])$	-0.084	1.00000	(-1.059,0.892)
$\mu([-]:[-]:[-]) - \mu([-]:[+]:[-])$	0.631	0.04461	(0.008,1.254)
$\mu([-]:[-]:[-]) - \mu([-]:[+]:[+])$	0.562	0.07304	(-0.026,1.151)
$\mu([-]:[-]:[-]) - \mu([+]:[-]:[-])$	1.067	0.22870	(-0.266,2.399)
$\mu([-]:[-]:[-]) - \mu([+]:[-]:[+])$	0.967	0.96920	(-1.864,3.798)
$\mu([-]:[-]:[-]) - \mu([+]:[+]:[-])$	1.739	0.00459	(0.329,3.150)
$\mu([-]:[-]:[-]) - \mu([+]:[+]:[+])$	1.561	0.00048	(0.458,2.664)
$\mu([-]:[-]:[+]) - \mu([-]:[+]:[-])$	0.715	0.30790	(-0.238,1.667)
$\mu([-]:[-]:[+]) - \mu([-]:[+]:[+])$	0.646	0.41130	(-0.284,1.576)
$\mu([-]:[-]:[+]) - \mu([+]:[-]:[-])$	1.150	0.29270	(-0.365,2.665)
$\mu([-]:[-]:[+]) - \mu([+]:[-]:[+])$	1.050	0.95910	(-1.871,3.972)
$\mu([-]:[-]:[+]) - \mu([+]:[+]:[-])$	1.823	0.01141	(0.239,3.407)
$\mu([-]:[-]:[+]) - \mu([+]:[+]:[+])$	1.644	0.00386	(0.327,2.962)
$\mu([-]:[+]:[-]) - \mu([-]:[+]:[+])$	-0.069	0.99990	(-0.618,0.480)
$\mu([-]:[+]:[-]) - \mu([+]:[-]:[-])$	0.436	0.97410	(-0.880,1.751)
$\mu([-]:[+]:[-]) - \mu([+]:[-]:[+])$	0.336	1.00000	(-2.488,3.159)
$\mu([-]:[+]:[-]) - \mu([+]:[+]:[-])$	1.108	0.23670	(-0.286,2.503)
$\mu([-]:[+]:[-]) - \mu([+]:[+]:[+])$	0.930	0.15470	(-0.153,2.012)
$\mu([-]:[+]:[+]) - \mu([+]:[-]:[-])$	0.504	0.93890	(-0.795,1.804)
$\mu([-]:[+]:[+]) - \mu([+]:[-]:[+])$	0.404	0.99990	(-2.411,3.220)
$\mu([-]:[+]:[+]) - \mu([+]:[+]:[-])$	1.177	0.16060	(-0.202,2.556)
$\mu([-]:[+]:[+]) - \mu([+]:[+]:[+])$	0.998	0.08377	(-0.064,2.061)
$\mu([+]:[-]:[-]) - \mu([+]:[-]:[+])$	-0.100	1.00000	(-3.159,2.959)
$\mu([+]:[-]:[-]) - \mu([+]:[+]:[-])$	0.673	0.95330	(-1.153,2.498)
$\mu([+]:[-]:[-]) - \mu([+]:[+]:[+])$	0.494	0.98260	(-1.106,2.094)
$\mu([+]:[-]:[+]) - \mu([+]:[+]:[-])$	0.773	0.99510	(-2.321,3.866)
$\mu([+]:[-]:[+]) - \mu([+]:[+]:[+])$	0.594	0.99880	(-2.372,3.560)
$\mu([+]:[+]:[-]) - \mu([+]:[+]:[+])$	-0.179	1.00000	(-1.844,1.486)

Table S5. (bla_{SHV}: bla_{CTX-M}: bla_{OXA}): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by bla_{SHV}, bla_{CTX-M}, and bla_{OXA} Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value. p-value<0.05 is significant

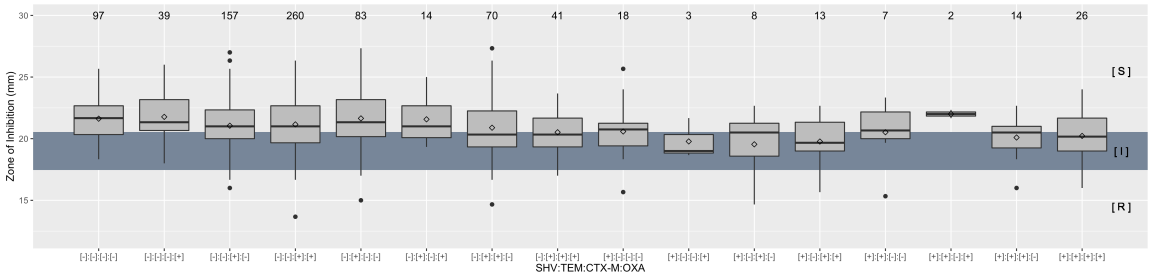

Figure S5. (*bla*$_{SHV}$: *bla*$_{TEM}$: *bla*$_{CTX-M}$: *bla*$_{OXA}$): Distributions of Zone of Inhibition (mm) Measurements by Resistance Gene Combination. Resistance gene presence is indicated by $[+]$ and resistance gene absence is indicated by $[-]$. The diamond $(\diamond)$ indicates the average zone of inhibition measurement for each condition and the number above each boxplot indicates the number of samples under that condition. The CLSI regions for resistance classification are labeled: susceptible $([\mathbf{S}])$, intermediate $([\mathbf{I}])$, and Resistant $([\mathbf{R}])$.

bla_{SHV}: bla_{TEM}: bla_{CTX-M}: bla_{OXA}	Difference	p-value	95% CI
$\mu([-]:[-]:[-]:[-]) - \mu([-]:[-]:[-]:[+])$	-0.147	1.00000	(-1.487,1.193)
$\mu([-]:[-]:[-]:[-]) - \mu([-]:[-]:[+]:[-])$	0.570	0.73930	(-0.343,1.483)
$\mu([-]:[-]:[-]:[-]) - \mu([-]:[-]:[+]:[+])$	0.464	0.88010	(-0.376,1.305)
$\mu([-]:[-]:[-]:[-]) - \mu([-]:[+]:[-]:[-])$	-0.025	1.00000	(-1.081,1.032)
$\mu([-]:[-]:[-]:[-]) - \mu([-]:[+]:[-]:[+])$	0.051	1.00000	(-1.970,2.071)
$\mu([-]:[-]:[-]:[-]) - \mu([-]:[+]:[+]:[-])$	0.732	0.65270	(-0.377,1.840)
$\mu([-]:[-]:[-]:[-]) - \mu([-]:[+]:[+]:[+])$	1.102	0.23160	(-0.215,2.418)
$\mu([-]:[-]:[-]:[-]) - \mu([+]:[-]:[-]:[-])$	1.039	0.84620	(-0.775,2.852)
$\mu([-]:[-]:[-]:[-]) - \mu([+]:[-]:[-]:[+])$	1.844	0.97980	(-2.298,5.987)
$\mu([-]:[-]:[-]:[-]) - \mu([+]:[-]:[+]:[-])$	2.080	0.30360	(-0.519,4.680)
$\mu([-]:[-]:[-]:[-]) - \mu([+]:[-]:[+]:[+])$	1.853	0.15190	(-0.234,3.940)
$\mu([-]:[-]:[-]:[-]) - \mu([+]:[+]:[-]:[-])$	1.098	0.99350	(-1.668,3.864)
$\mu([-]:[-]:[-]:[-]) - \mu([+]:[+]:[-]:[+])$	-0.378	1.00000	(-5.426,4.670)
$\mu([-]:[-]:[-]:[-]) - \mu([+]:[+]:[+]:[-])$	1.527	0.40620	(-0.494,3.547)
$\mu([-]:[-]:[-]:[-]) - \mu([+]:[+]:[+]:[+])$	1.398	0.14160	(-0.163,2.958)
$\mu([-]:[-]:[-]:[+]) - \mu([-]:[-]:[+]:[-])$	0.717	0.85570	(-0.547,1.982)
$\mu([-]:[-]:[-]:[+]) - \mu([-]:[-]:[+]:[+])$	0.612	0.94020	(-0.602,1.825)
$\mu([-]:[-]:[-]:[+]) - \mu([-]:[+]:[-]:[-])$	0.123	1.00000	(-1.249,1.495)
$\mu([-]:[-]:[-]:[+]) - \mu([-]:[+]:[-]:[+])$	0.198	1.00000	(-2.004,2.400)
$\mu([-]:[-]:[-]:[+]) - \mu([-]:[+]:[+]:[-])$	0.879	0.74440	(-0.533,2.291)
$\mu([-]:[-]:[-]:[+]) - \mu([-]:[+]:[+]:[+])$	1.249	0.32570	(-0.332,2.830)
$\mu([-]:[-]:[-]:[+]) - \mu([+]:[-]:[-]:[-])$	1.186	0.81580	(-0.828,3.200)
$\mu([-]:[-]:[-]:[+]) - \mu([+]:[-]:[-]:[+])$	1.991	0.96670	(-2.243,6.226)
$\mu([-]:[-]:[-]:[+]) - \mu([+]:[-]:[+]:[-])$	2.228	0.27900	(-0.515,4.970)
$\mu([-]:[-]:[-]:[+]) - \mu([+]:[-]:[+]:[+])$	2.000	0.15720	(-0.263,4.263)
$\mu([-]:[-]:[-]:[+]) - \mu([+]:[+]:[-]:[-])$	1.245	0.98570	(-1.655,4.146)
$\mu([-]:[-]:[-]:[+]) - \mu([+]:[+]:[-]:[+])$	-0.231	1.00000	(-5.354,4.893)
$\mu([-]:[-]:[-]:[+]) - \mu([+]:[+]:[+]:[-])$	1.674	0.39490	(-0.528,3.876)
$\mu([-]:[-]:[-]:[+]) - \mu([+]:[+]:[+]:[+])$	1.545	0.18700	(-0.244,3.334)
$\mu([-]:[-]:[+]:[-]) - \mu([-]:[-]:[+]:[+])$	-0.106	1.00000	(-0.820,0.609)
$\mu([-]:[-]:[+]:[-]) - \mu([-]:[+]:[-]:[-])$	-0.595	0.74980	(-1.554,0.364)
$\mu([-]:[-]:[+]:[-]) - \mu([-]:[+]:[-]:[+])$	-0.519	0.99990	(-2.491,1.452)
$\mu([-]:[-]:[+]:[-]) - \mu([-]:[+]:[+]:[-])$	0.162	1.00000	(-0.854,1.177)
$\mu([-]:[-]:[+]:[-]) - \mu([-]:[+]:[+]:[+])$	0.532	0.98580	(-0.708,1.771)
$\mu([-]:[-]:[+]:[-]) - \mu([+]:[-]:[-]:[-])$	0.469	0.99990	(-1.290,2.227)
$\mu([-]:[-]:[+]:[-]) - \mu([+]:[-]:[-]:[+])$	1.274	0.99960	(-2.845,5.393)
$\mu([-]:[-]:[+]:[-]) - \mu([+]:[-]:[+]:[-])$	1.510	0.81430	(-1.051,4.072)
$\mu([-]:[-]:[+]:[-]) - \mu([+]:[-]:[+]:[+])$	1.283	0.72900	(-0.757,3.322)
$\mu([-]:[-]:[+]:[-]) - \mu([+]:[+]:[-]:[-])$	0.528	1.00000	(-2.202,3.258)
$\mu([-]:[-]:[+]:[-]) - \mu([+]:[+]:[-]:[+])$	-0.948	1.00000	(-5.977,4.081)
$\mu([-]:[-]:[+]:[-]) - \mu([+]:[+]:[+]:[-])$	0.957	0.95620	(-1.014,2.928)
$\mu([-]:[-]:[+]:[-]) - \mu([+]:[+]:[+]:[+])$	0.828	0.87870	(-0.669,2.324)
$\mu([-]:[-]:[+]:[+]) - \mu([-]:[+]:[-]:[-])$	-0.489	0.88540	(-1.380,0.402)
$\mu([-]:[-]:[+]:[+]) - \mu([-]:[+]:[-]:[+])$	-0.414	1.00000	(-2.353,1.525)
$\mu([-]:[-]:[+]:[+]) - \mu([-]:[+]:[+]:[-])$	0.267	0.99990	(-0.684,1.219)
$\mu([-]:[-]:[+]:[+]) - \mu([-]:[+]:[+]:[+])$	0.637	0.90240	(-0.550,1.825)
$\mu([-]:[-]:[+]:[+]) - \mu([+]:[-]:[-]:[-])$	0.574	0.99910	(-1.148,2.297)
$\mu([-]:[-]:[+]:[+]) - \mu([+]:[-]:[-]:[+])$	1.380	0.99900	(-2.724,5.483)
$\mu([-]:[-]:[+]:[+]) - \mu([+]:[-]:[+]:[-])$	1.616	0.70970	(-0.921,4.153)
$\mu([-]:[-]:[+]:[+]) - \mu([+]:[-]:[+]:[+])$	1.388	0.57160	(-0.620,3.397)

$\mu([-]:[-]:[+]:[+]) - \mu([+]:[+]:[-]:[-])$	0.634	1.00000	(-2.073,3.341)
$\mu([-]:[-]:[+]:[+]) - \mu([+]:[+]:[-]:[+])$	-0.842	1.00000	(-5.859,4.174)
$\mu([-]:[-]:[+]:[+]) - \mu([+]:[+]:[+]:[-])$	1.062	0.88650	(-0.876,3.001)
$\mu([-]:[-]:[+]:[+]) - \mu([+]:[+]:[+]:[+])$	0.933	0.69750	(-0.520,2.387)
$\mu([-]:[+]:[-]:[-]) - \mu([-]:[+]:[-]:[+])$	0.075	1.00000	(-1.967,2.117)
$\mu([-]:[+]:[-]:[-]) - \mu([-]:[+]:[+]:[-])$	0.756	0.65450	(-0.391,1.903)
$\mu([-]:[+]:[-]:[-]) - \mu([-]:[+]:[+]:[+])$	1.126	0.23520	(-0.223,2.475)
$\mu([-]:[+]:[-]:[-]) - \mu([+]:[-]:[-]:[-])$	1.063	0.83540	(-0.774,2.901)
$\mu([-]:[+]:[-]:[-]) - \mu([+]:[-]:[-]:[+])$	1.869	0.97770	(-2.284,6.022)
$\mu([-]:[+]:[-]:[-]) - \mu([+]:[-]:[+]:[-])$	2.105	0.29460	(-0.511,4.721)
$\mu([-]:[+]:[-]:[-]) - \mu([+]:[-]:[+]:[+])$	1.877	0.14790	(-0.231,3.985)
$\mu([-]:[+]:[-]:[-]) - \mu([+]:[+]:[-]:[-])$	1.123	0.99230	(-1.659,3.904)
$\mu([-]:[+]:[-]:[-]) - \mu([+]:[+]:[-]:[+])$	-0.353	1.00000	(-5.410,4.703)
$\mu([-]:[+]:[-]:[-]) - \mu([+]:[+]:[+]:[-])$	1.551	0.39610	(-0.490,3.593)
$\mu([-]:[+]:[-]:[-]) - \mu([+]:[+]:[+]:[+])$	1.422	0.14170	(-0.166,3.010)
$\mu([-]:[+]:[-]:[+]) - \mu([-]:[+]:[+]:[-])$	0.681	0.99920	(-1.388,2.750)
$\mu([-]:[+]:[-]:[+]) - \mu([-]:[+]:[+]:[+])$	1.051	0.95990	(-1.136,3.239)
$\mu([-]:[+]:[-]:[+]) - \mu([+]:[-]:[-]:[-])$	0.988	0.99430	(-1.530,3.506)
$\mu([-]:[+]:[-]:[+]) - \mu([+]:[-]:[-]:[+])$	1.794	0.99320	(-2.702,6.290)
$\mu([-]:[+]:[-]:[+]) - \mu([+]:[-]:[+]:[-])$	2.030	0.68280	(-1.102,5.162)
$\mu([-]:[+]:[-]:[+]) - \mu([+]:[-]:[+]:[+])$	1.802	0.64750	(-0.920,4.524)
$\mu([-]:[+]:[-]:[+]) - \mu([+]:[+]:[-]:[-])$	1.048	0.99940	(-2.224,4.319)
$\mu([-]:[+]:[-]:[+]) - \mu([+]:[+]:[-]:[+])$	-0.429	1.00000	(-5.771,4.913)
$\mu([-]:[+]:[-]:[+]) - \mu([+]:[+]:[+]:[-])$	1.476	0.87940	(-1.195,4.147)
$\mu([-]:[+]:[-]:[+]) - \mu([+]:[+]:[+]:[+])$	1.347	0.84200	(-0.996,3.690)
$\mu([-]:[+]:[+]:[-]) - \mu([-]:[+]:[+]:[+])$	0.370	0.99990	(-1.020,1.760)
$\mu([-]:[+]:[+]:[-]) - \mu([+]:[-]:[-]:[-])$	0.307	1.00000	(-1.560,2.175)
$\mu([-]:[+]:[+]:[-]) - \mu([+]:[-]:[-]:[+])$	1.113	0.99990	(-3.054,5.279)
$\mu([-]:[+]:[+]:[-]) - \mu([+]:[-]:[+]:[-])$	1.349	0.93270	(-1.289,3.986)
$\mu([-]:[+]:[+]:[-]) - \mu([+]:[-]:[+]:[+])$	1.121	0.91700	(-1.013,3.255)
$\mu([-]:[+]:[+]:[-]) - \mu([+]:[+]:[-]:[-])$	0.367	1.00000	(-2.435,3.168)
$\mu([-]:[+]:[+]:[-]) - \mu([+]:[+]:[-]:[+])$	-1.110	1.00000	(-6.177,3.958)
$\mu([-]:[+]:[+]:[-]) - \mu([+]:[+]:[+]:[-])$	0.795	0.99540	(-1.274,2.864)
$\mu([-]:[+]:[+]:[-]) - \mu([+]:[+]:[+]:[+])$	0.666	0.99090	(-0.957,2.289)
$\mu([-]:[+]:[+]:[+]) - \mu([+]:[-]:[-]:[-])$	-0.063	1.00000	(-2.061,1.935)
$\mu([-]:[+]:[+]:[+]) - \mu([+]:[-]:[-]:[+])$	0.743	1.00000	(-3.484,4.969)
$\mu([-]:[+]:[+]:[+]) - \mu([+]:[-]:[+]:[-])$	0.979	0.99790	(-1.753,3.710)
$\mu([-]:[+]:[+]:[+]) - \mu([+]:[-]:[+]:[+])$	0.751	0.99900	(-1.498,3.000)
$\mu([-]:[+]:[+]:[+]) - \mu([+]:[+]:[-]:[-])$	-0.003	1.00000	(-2.894,2.887)
$\mu([-]:[+]:[+]:[+]) - \mu([+]:[+]:[-]:[+])$	-1.480	0.99980	(-6.597,3.638)
$\mu([-]:[+]:[+]:[+]) - \mu([+]:[+]:[+]:[-])$	0.425	1.00000	(-1.762,2.613)
$\mu([-]:[+]:[+]:[+]) - \mu([+]:[+]:[+]:[+])$	0.296	1.00000	(-1.476,2.068)
$\mu([+]:[-]:[-]:[-]) - \mu([+]:[-]:[-]:[+])$	0.806	1.00000	(-3.601,5.213)
$\mu([+]:[-]:[-]:[-]) - \mu([+]:[-]:[+]:[-])$	1.042	0.99850	(-1.961,4.045)
$\mu([+]:[-]:[-]:[-]) - \mu([+]:[-]:[+]:[+])$	0.814	0.99950	(-1.758,3.386)
$\mu([+]:[-]:[-]:[-]) - \mu([+]:[+]:[-]:[-])$	0.060	1.00000	(-3.088,3.207)
$\mu([+]:[-]:[-]:[-]) - \mu([+]:[+]:[-]:[+])$	-1.417	0.99990	(-6.684,3.851)
$\mu([+]:[-]:[-]:[-]) - \mu([+]:[+]:[+]:[-])$	0.488	1.00000	(-2.030,3.006)
$\mu([+]:[-]:[-]:[-]) - \mu([+]:[+]:[+]:[+])$	0.359	1.00000	(-1.808,2.526)

$\mu([+]:[-]:[-]:[+]) - \mu([+]:[-]:[+]:[-])$	0.236	1.00000	(-4.548,5.020)
$\mu([+]:[-]:[-]:[+]) - \mu([+]:[-]:[+]:[+])$	0.009	1.00000	(-4.518,4.535)
$\mu([+]:[-]:[-]:[+]) - \mu([+]:[+]:[-]:[-])$	-0.746	1.00000	(-5.623,4.131)
$\mu([+]:[-]:[-]:[+]) - \mu([+]:[+]:[-]:[+])$	-2.222	0.99860	(-8.673,4.229)
$\mu([+]:[-]:[-]:[+]) - \mu([+]:[+]:[+]:[-])$	-0.317	1.00000	(-4.813,4.179)
$\mu([+]:[-]:[-]:[+]) - \mu([+]:[+]:[+]:[+])$	-0.447	1.00000	(-4.756,3.862)
$\mu([+]:[-]:[+]:[-]) - \mu([+]:[-]:[+]:[+])$	-0.228	1.00000	(-3.403,2.948)
$\mu([+]:[-]:[+]:[-]) - \mu([+]:[+]:[-]:[-])$	-0.982	0.99990	(-4.640,2.675)
$\mu([+]:[-]:[+]:[-]) - \mu([+]:[+]:[-]:[+])$	-2.458	0.98190	(-8.045,3.129)
$\mu([+]:[-]:[+]:[-]) - \mu([+]:[+]:[+]:[-])$	-0.554	1.00000	(-3.686,2.578)
$\mu([+]:[-]:[+]:[-]) - \mu([+]:[+]:[+]:[+])$	-0.683	1.00000	(-3.540,2.174)
$\mu([+]:[-]:[+]:[+]) - \mu([+]:[+]:[-]:[-])$	-0.755	1.00000	(-4.068,2.558)
$\mu([+]:[-]:[+]:[+]) - \mu([+]:[+]:[-]:[+])$	-2.231	0.98960	(-7.598,3.137)
$\mu([+]:[-]:[+]:[+]) - \mu([+]:[+]:[+]:[-])$	-0.326	1.00000	(-3.048,2.396)
$\mu([+]:[-]:[+]:[+]) - \mu([+]:[+]:[+]:[+])$	-0.455	1.00000	(-2.856,1.945)
$\mu([+]:[+]:[-]:[-]) - \mu([+]:[+]:[-]:[+])$	-1.476	1.00000	(-7.142,4.190)
$\mu([+]:[+]:[-]:[-]) - \mu([+]:[+]:[+]:[-])$	0.429	1.00000	(-2.843,3.700)
$\mu([+]:[+]:[-]:[-]) - \mu([+]:[+]:[+]:[+])$	0.299	1.00000	(-2.710,3.309)
$\mu([+]:[+]:[-]:[+]) - \mu([+]:[+]:[+]:[-])$	1.905	0.99800	(-3.437,7.247)
$\mu([+]:[+]:[-]:[+]) - \mu([+]:[+]:[+]:[+])$	1.776	0.99870	(-3.410,6.961)
$\mu([+]:[+]:[+]:[-]) - \mu([+]:[+]:[+]:[+])$	-0.129	1.00000	(-2.472,2.214)

Table S6. (*bla*$_{SHV}$: *bla*$_{TEM}$: *bla*$_{CTX-M}$: *bla*$_{OXA}$): Test for Mean Differences Between Zone of Inhibition (mm) Measurements by *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{OXA}$ Resistance Gene Combinations. The 95% confidence intervals from Tukey's honest significance test with the associated *p*-value.

Chapter 3 Supplementals:

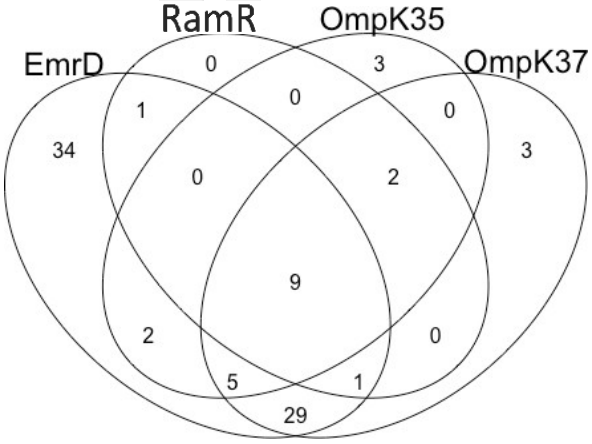

Figure S6. Distribution of the non-β-lactamase genes emrD, RamR, OmpK35, and OmpK37 in 95 ESBL+ isolates

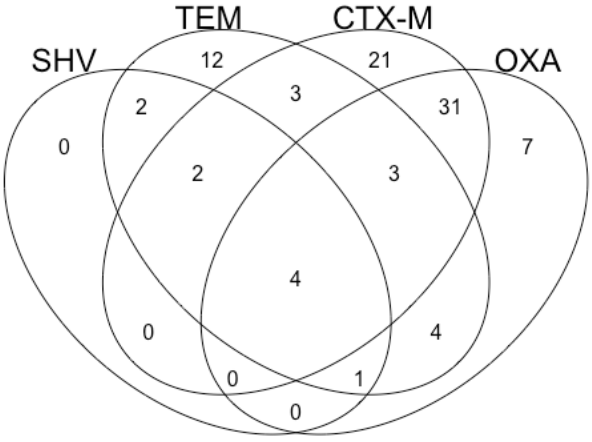

Figure S7. Distribution of the β-lactamase genes SHV, TEM, CTX-M, and OXA in 95 ESBL+ isolates

Table S6. Genomic sequence data: Single gene association with c/t resistance

Mean ZI(ARO[-])	Mean ZI(ARO[+])	Number of isolates [-][+]	p-value	Gene
20.17508418	20.30555556	[99][24]	0.793	patA
18.96296296	20.29824561	[9][114]	0.076	emrB
20.11428571	20.7037037	[105][18]	0.289	tet(A)
20.10032362	20.71666667	[103][20]	0.247	emrD

20.23478261	19.70833333	[115][8]	0.509	mphA
20.08099688	21	[107][16]	0.115	ErmB
20.24210526	20.05952381	[95][28]	0.697	sul1
20.19314642	20.25	[107][16]	0.923	sul2
20.1218638	20.44444444	[93][30]	0.481	acrD
19.40740741	20.26315789	[9][114]	0.257	AcrF
20.26315789	20.09929078	[76][47]	0.686	CRP
20.19710145	20.25	[115][8]	0.947	AcrS
20.11111111	20.22222222	[24][99]	0.823	H-NS
20.1037037	20.25641026	[45][78]	0.709	mdtB
20.18556701	20.25641026	[97][26]	0.883	mdtC
19.16666667	20.2920354	[10][113]	0.116	mdtF
19.91145833	20.51412429	[64][59]	0.124	TEM-4
20.09009009	20.36734694	[74][49]	0.490	mdtM
20.19365079	20.24074074	[105][18]	0.933	mdtH
20.16666667	20.21052632	[28][95]	0.926	MdtK
20.29230769	20.09770115	[65][58]	0.622	mdfA
20.12790698	20.36936937	[86][37]	0.574	mdtG
20.49152542	19.93229167	[59][64]	0.154	OXA-1
20.28787879	19.46153846	[110][13]	0.195	OXA-2
20.03773585	21.21568627	[106][17]	0.037	CTX-M-14
20.29931973	20.13513514	[49][74]	0.683	CTX-M-15
20.13149847	20.73809524	[109][14]	0.327	AAC(3)-IIa
20.26595745	19.98850575	[94][29]	0.550	AAC(3)-IIc
20.24107143	19.78787879	[112][11]	0.511	aadA5
20.02409639	20.56666667	[83][40]	0.195	APH(6)-Id
20.16374269	20.66666667	[114][9]	0.506	floR
20.23529412	20.17592593	[51][72]	0.882	vgaC
20.13782051	20.54385965	[104][19]	0.456	arnA
20.28695652	18.95833333	[115][8]	0.094	bacA
20.09057971	20.52688172	[92][31]	0.335	gyrA
19	20.29532164	[9][114]	0.085	gyrB
20.2969697	19.38461538	[110][13]	0.153	gyrB

20.27619048	19.75925926	[105][18]	0.353	parC conferring
20.1152648	20.77083333	[107][16]	0.262	parE
20.1951952	20.25	[111][12]	0.934	16S rRNA (rrsC)
20.56349206	20.01234568	[42][81]	0.183	EF-Tu mutantation
19.9929078	20.87356322	[94][29]	0.056	16S rRNA mutation
20.1025641	20.37037037	[78][45]	0.512	marR
20.2173913	19.95833333	[115][8]	0.746	ramR
20.15915916	20.58333333	[111][12]	0.522	soxR
20.22629969	20	[109][14]	0.715	Omp36
20.23893805	19.76666667	[113][10]	0.512	folP
20.23123123	19.91666667	[111][12]	0.635	ompF
20.37037037	20.15277778	[27][96]	0.647	16S rRNA mutation
20.09785933	21	[109][14]	0.144	16S rRNA mutation
19.16666667	20.2920354	[10][113]	0.116	mdtO
20.19191919	20.23611111	[99][24]	0.929	PmrC
20.18148148	20.25252525	[90][33]	0.873	PmrE
20.27037037	20.01010101	[90][33]	0.558	PhoP
20.2172619	20.03030303	[112][11]	0.786	nfsA
20.10897436	20.70175439	[104][19]	0.275	murA
20.19710145	20.25	[115][8]	0.947	acrR
20.14285714	20.78787879	[112][11]	0.349	mgrB
20.42767296	20.02857143	[53][70]	0.315	Mrx
20.22222222	20.15873016	[81][42]	0.878	kdpE
20.09621993	20.58974359	[97][26]	0.305	UhpT
20.12753623	21.25	[115][8]	0.158	UhpA
20.21021021	20.11111111	[111][12]	0.881	PtsI
20.27987421	19.70588235	[106][17]	0.313	oqxB
20.08130081	20.2601626	[41][82]	0.668	parC
19.16666667	20.35514019	[16][107]	0.041	msbA
20.33333333	**19.07692308**	**[110][13]**	**0.048***	**OmpK35**
20.17109145	20.53333333	[113][10]	0.615	OmpK36
20.00854701	20.28968254	[39][84]	0.506	emrE

19.17777778	20.34259259	[15][108]	0.051	fabI
20.17592593	20.37777778	[108][15]	0.737	fabG
20.18348624	20.33333333	[109][14]	0.809	OmpC
20.35238095	**19.31481481**	**[105][18]**	**0.061***	**OmpK37**
19.91666667	20.60130719	[72][51]	0.085	LamB
20.37254902	20.17295597	[17][106]	0.726	mipA
20.34444444	20.06349206	[60][63]	0.475	EampC
20.29357798	19.47619048	[109][14]	0.186	gyrA

Table S7. Sequence data Selected two-gene interactions, EmrD with other genes. Column labeled “number in isolates” first brackets shows number of isolates lacking both genes, second brackets shows number of isolates that have both genes.

Gene 1	Gene 2	number in isolates	p value
EmrD	mphA	[122][1]	0.815
EmrD	ErmB	[110][13]	0.124
EmrD	sul1	[120][3]	0.124
EmrD	sul2	[122][1]	0.124
EmrD	acrD	[114][9]	0.728
EmrD	AcrF	[103][20]	0.247
EmrD	CRP	[113][10]	0.364
EmrD	AcrS	[118][5]	0.364
EmrD	H-NS	[103][20]	0.247
EmrD	mdtB	[105][18]	0.099
EmrD	mdtC	[113][10]	0.173
EmrD	mdtF	[104][19]	0.088
EmrD	TEM-4	[105][18]	0.099
EmrD	mdtM	[112][11]	0.077
EmrD	mdtH	[117][6]	0.077*
EmrD	MdtK	[107][16]	0.135
EmrD	mdfA	[114][9]	0.933
EmrD	mdtG	[105][18]	0.099
EmrD	OXA-1	[116][7]	0.099*
EmrD	OXA-2	[121][2]	0.099*
EmrD	CTX-M-14	[109][14]	0.057
EmrD	CTX-M-15	[113][10]	0.267

EmrD	AAC(3)-IIa	[114][9]	0.650
EmrD	AAC(3)-IIc	[119][4]	0.650
EmrD	aadA5	[123][0]	0.650
EmrD	APH(6)-Id	[111][12]	0.522
EmrD	floR	[121][2]	0.522
EmrD	vgaC	[116][7]	0.522
EmrD	arnA	[115][8]	0.309
EmrD	bacA	[118][5]	0.309
EmrD	gyrA	[118][5]	0.309
EmrD	gyrB	[104][19]	0.088
EmrD	gyrB	[121][2]	0.088*
EmrD	parC	[119][4]	0.088*
EmrD	parE	[115][8]	0.992
EmrD	16S rRNA (rrsC) mutation	[120][3]	0.992
EmrD	EF-Tu mutants	[116][7]	0.992
EmrD	16S rRNA (rrsH)	[108][15]	0.164
EmrD	marR mutant	[106][17]	0.072
EmrD	**ramR mutants**	**[121][2]**	**0.072***
EmrD	soxR	[115][8]	0.428
EmrD	Omp36	[117][6]	0.428
EmrD	folP	[117][6]	0.428
EmrD	ompF	[118][5]	0.428
EmrD	16S rRNA (rrsB) mutation	[114][9]	0.728
EmrD	16S rRNA mutation	[110][13]	0.037
EmrD	mdtO	[104][19]	0.088
EmrD	PmrC	[117][6]	0.088
EmrD	PmrE	[114][9]	0.300
EmrD	PhoP	[113][10]	0.841
EmrD	nfsA	[117][6]	0.841
EmrD	murA	[117][6]	0.841
EmrD	acrR	[119][4]	0.841
EmrD	mgrB	[117][6]	0.841
EmrD	Mrx	[109][14]	0.446
EmrD	kdpE	[113][10]	0.364

EmrD	UhpT	[111][12]	0.180
EmrD	UhpA	[117][6]	0.180
EmrD	PtsI	[117][6]	0.180
EmrD	oqxB	[121][2]	0.180
EmrD	parC	[107][16]	0.135
EmrD	msbA	[104][19]	0.088
EmrD	OmpK35	[120][3]	0.088*
EmrD	OmpK36	[120][3]	0.088
EmrD	emrE	[104][19]	0.088
EmrD	fabI	[104][19]	0.088
EmrD	fabG	[116][7]	0.088*
EmrD	OmpC	[117][6]	0.088*
EmrD	OmpK37	[119][4]	0.088*
EmrD	LamB	[104][19]	0.275
EmrD	mipA	[103][20]	0.247
EmrD	ampC	[115][8]	0.831
EmrD	gyrA	[121][2]	0.831

Table S8. OmpK35 gene interactions from genomic sequence data. Column labeled “number in isolates” first brackets shows number of isolates lacking both genes, second brackets shows number of isolates that have both genes.

Gene1:Gene2	Number in Isolates [-] [+]	p-value
OmpK35:OmpK36	[121][2]	0.676
OmpK35:EmrE	[117][6]	0.676
OmpK35:FabI	[118][5]	0.676
OmpK35:FabG	[117][6]	0.676
OmpK35:OmpC	[118][5]	0.676
OmpK35:OmpK37	[112][11]	0.054*
OmpK35:LamB	[110][13]	0.048*
OmpK35:mipA	[118][5]	0.048*
OmpK35:AmpC	[117][6]	0.048*
OmpK35:gyrA	[112][11]	0.054*

Table S9. Gene interactions between all genes (beta lactamases and non-beta lactamases) from PCR screen, using Tuckey’s Honest Significant Differences test.

Gene1:Gene 2	Condition	Sample Size(s)	Difference	p-value	CI (95%)
EmrD:ramR	E([-]:[-])-E([-]:[+])	[13][2]	-0.96	9.62e-01	(-6.14,4.21)

	E([-]:[-])-E([+]:[-])	[13][70]	-1.38	3.03e-01	(-3.44,0.68)
	E([-]:[-])-E([+]:[+])	[13][10]	-0.29	9.93e-01	(-3.16,2.57)
	E([-]:[+])-E([+]:[-])	[2][70]	-0.42	9.96e-01	(-5.30,4.47)
	E([-]:[+])-E([+]:[+])	[2][10]	0.67	9.87e-01	(-4.61,5.94)
	E([+]:[-])-E([+]:[+])	[70][10]	1.08	6.09e-01	(-1.22,3.39)
EmrD:OmpK35	E([-]:[-])-E([-]:[+])	[10][5]	2.05	4.42e-01	(-1.53,5.63)
	E([-]:[-])-E([+]:[-])	[10][65]	-0.80	7.81e-01	(-3.02,1.42)
	E([-]:[-])-E([+]:[+])	[10][15]	1.17	6.60e-01	(-1.50,3.84)
	E([-]:[+])-E([+]:[-])	[5][65]	-2.85	7.31e-02	(-5.88,0.18)
	E([-]:[+])-E([+]:[+])	[5][15]	-0.88	9.04e-01	(-4.25,2.50)
	E([+]:[-])-E([+]:[+])	[65][15]	1.97	3.47e-02	(0.10,3.85)
EmrD:OmpK37	E([-]:[-])-E([-]:[+])	[10][5]	-2.15	4.35e-01	(-5.87,1.57)
	E([-]:[-])-E([+]:[-])	[10][37]	-1.84	2.01e-01	(-4.26,0.58)
	E([-]:[-])-E([+]:[+])	[10][43]	-1.83	1.95e-01	(-4.21,0.56)
	E([-]:[+])-E([+]:[-])	[5][37]	0.31	9.94e-01	(-2.93,3.55)
	E([-]:[+])-E([+]:[+])	[5][43]	0.32	9.93e-01	(-2.89,3.53)
	E([+]:[-])-E([+]:[+])	[37][43]	0.01	1.00e+00	(-1.51,1.54)
EmrD:SHV	E([-]:[-])-E([-]:[+])	[14][1]	-0.54	9.97e-01	(-7.64,6.57)
	E([-]:[-])-E([+]:[-])	[14][72]	-1.20	4.03e-01	(-3.21,0.81)
	E([-]:[-])-E([+]:[+])	[14][8]	-0.70	9.31e-01	(-3.75,2.34)
	E([-]:[+])-E([+]:[-])	[1][72]	-0.66	9.94e-01	(-7.58,6.25)
	E([-]:[+])-E([+]:[+])	[1][8]	-0.17	1.00e+00	(-7.45,7.12)
	E([+]:[-])-E([+]:[+])	[72][8]	0.50	9.57e-01	(-2.06,3.06)
EmrD:TEM	E([-]:[-])-E([-]:[+])	[12][3]	1.04	9.27e-01	(-3.39,5.47)
	E([-]:[-])-E([+]:[-])	[12][52]	-0.93	6.86e-01	(-3.13,1.27)
	E([-]:[-])-E([+]:[+])	[12][28]	-0.86	7.76e-01	(-3.23,1.51)
	E([-]:[+])-E([+]:[-])	[3][52]	-1.97	5.87e-01	(-6.05,2.10)
	E([-]:[+])-E([+]:[+])	[3][28]	-1.90	6.31e-01	(-6.07,2.27)
	E([+]:[-])-E([+]:[+])	[52][28]	0.07	1.00e+00	(-1.54,1.68)
EmrD:CTX-M	E([-]:[-])-E([-]:[+])	[4][11]	-0.68	9.70e-01	(-4.69,3.32)
	E([-]:[-])-E([+]:[-])	[4][27]	-1.86	5.51e-01	(-5.53,1.82)
	E([-]:[-])-E([+]:[+])	[4][53]	-1.49	6.92e-01	(-5.05,2.07)
	E([-]:[+])-E([+]:[-])	[11][27]	-1.18	5.94e-01	(-3.63,1.28)
	E([-]:[+])-E([+]:[+])	[11][53]	-0.81	7.88e-01	(-3.08,1.46)
	E([+]:[-])-E([+]:[+])	[27][53]	0.37	9.34e-01	(-1.25,1.99)
EmrD:OXA	E([-]:[-])-E([-]:[+])	[7][8]	-0.49	9.84e-01	(-4.04,3.06)
	E([-]:[-])-E([+]:[-])	[7][38]	-1.23	6.65e-01	(-4.06,1.59)
	E([-]:[-])-E([+]:[+])	[7][42]	-1.51	4.98e-01	(-4.31,1.29)
	E([-]:[+])-E([+]:[-])	[8][38]	-0.74	8.87e-01	(-3.41,1.93)
	E([-]:[+])-E([+]:[+])	[8][42]	-1.02	7.47e-01	(-3.67,1.63)
	E([+]:[-])-E([+]:[+])	[38][42]	-0.28	9.65e-01	(-1.81,1.26)
ramR:OmpK35	E([-]:[-])-E([-]:[+])	[73][10]	2.63	1.24e-02	(0.43,4.84)
	E([-]:[-])-E([+]:[-])	[73][2]	-0.98	9.47e-01	(-5.66,3.70)
	E([-]:[-])-E([+]:[+])	[73][10]	1.48	2.97e-01	(-0.72,3.69)
	E([-]:[+])-E([+]:[-])	[10][2]	-3.62	2.48e-01	(-8.68,1.44)
	E([-]:[+])-E([+]:[+])	[10][10]	-1.15	7.32e-01	(-4.07,1.77)
	E([+]:[-])-E([+]:[+])	[2][10]	2.47	5.81e-01	(-2.59,7.53)
tetR:OmpK37	E([-]:[-])-E([-]:[+])	[46][37]	-0.80	5.07e-01	(-2.30,0.70)
	E([-]:[-])-E([+]:[-])	[46][1]	-2.49	7.80e-01	(-9.37,4.39)
	E([-]:[-])-E([+]:[+])	[46][11]	0.66	8.73e-01	(-1.62,2.95)
	E([-]:[+])-E([+]:[-])	[37][1]	-1.69	9.19e-01	(-8.59,5.21)

	E([-]:[+])-E([+]:[+])	[37][11]	1.46	3.64e-01	(-0.88,3.80)
	E([+]:[-])-E([+]:[+])	[1][11]	3.15	6.54e-01	(-3.96,10.26)
tetR:SHV	E([-]:[-])-E([-]:[+])	[76][7]	0.27	9.94e-01	(-2.47,3.01)
	E([-]:[-])-E([+]:[-])	[76][10]	0.76	8.30e-01	(-1.57,3.09)
	E([-]:[-])-E([+]:[+])	[76][2]	0.89	9.66e-01	(-4.07,5.85)
	E([-]:[+])-E([+]:[-])	[7][10]	0.49	9.82e-01	(-2.93,3.90)
	E([-]:[+])-E([+]:[+])	[7][2]	0.62	9.91e-01	(-4.94,6.17)
	E([+]:[-])-E([+]:[+])	[10][2]	0.13	1.00e+00	(-5.23,5.50)
tetR:TEM	E([-]:[-])-E([-]:[+])	[58][25]	0.13	9.97e-01	(-1.52,1.79)
	E([-]:[-])-E([+]:[-])	[58][6]	1.19	7.23e-01	(-1.78,4.15)
	E([-]:[-])-E([+]:[+])	[58][6]	0.41	9.84e-01	(-2.56,3.38)
	E([-]:[+])-E([+]:[-])	[25][6]	1.05	8.18e-01	(-2.09,4.20)
	E([-]:[+])-E([+]:[+])	[25][6]	0.27	9.96e-01	(-2.87,3.42)
	E([+]:[-])-E([+]:[+])	[6][6]	-0.78	9.57e-01	(-4.77,3.22)
tetR:CTX-M	E([-]:[-])-E([-]:[+])	[26][57]	0.03	1.00e+00	(-1.59,1.66)
	E([-]:[-])-E([+]:[-])	[26][5]	-0.38	9.91e-01	(-3.73,2.98)
	E([-]:[-])-E([+]:[+])	[26][7]	1.61	4.80e-01	(-1.32,4.53)
	E([-]:[+])-E([+]:[-])	[57][5]	-0.41	9.87e-01	(-3.61,2.79)
	E([-]:[+])-E([+]:[+])	[57][7]	1.57	4.45e-01	(-1.18,4.32)
	E([+]:[-])-E([+]:[+])	[5][7]	1.98	5.72e-01	(-2.04,6.00)
tetR:OXA	E([-]:[-])-E([-]:[+])	[41][42]	-0.44	8.75e-01	(-1.95,1.08)
	E([-]:[-])-E([+]:[-])	[41][4]	0.40	9.92e-01	(-3.22,4.02)
	E([-]:[-])-E([+]:[+])	[41][8]	0.60	9.34e-01	(-2.07,3.28)
	E([-]:[+])-E([+]:[-])	[42][4]	0.83	9.31e-01	(-2.78,4.45)
	E([-]:[+])-E([+]:[+])	[42][8]	1.04	7.37e-01	(-1.62,3.71)
	E([+]:[-])-E([+]:[+])	[4][8]	0.21	9.99e-01	(-4.02,4.44)
OmpK35:OmpK37	E([-]:[-])-E([-]:[+])	[42][33]	-0.80	5.13e-01	(-2.30,0.71)
	E([-]:[-])-E([+]:[-])	[42][5]	2.79	8.65e-02	(-0.27,5.86)
	E([-]:[-])-E([+]:[+])	[42][15]	1.38	2.53e-01	(-0.56,3.33)
	E([-]:[+])-E([+]:[-])	[33][5]	3.59	1.67e-02	(0.48,6.70)
	E([-]:[+])-E([+]:[+])	[33][15]	2.18	2.88e-02	(0.16,4.19)
	E([+]:[-])-E([+]:[+])	[5][15]	-1.41	6.87e-01	(-4.75,1.93)
OmpK35:SHV	E([-]:[-])-E([-]:[+])	[69][6]	0.83	8.61e-01	(-1.94,3.61)
	E([-]:[-])-E([+]:[-])	[69][17]	2.41	3.20e-03	(0.64,4.17)
	E([-]:[-])-E([+]:[+])	[69][3]	0.72	9.61e-01	(-3.12,4.57)
	E([-]:[+])-E([+]:[-])	[6][17]	1.57	5.47e-01	(-1.52,4.67)
	E([-]:[+])-E([+]:[+])	[6][3]	-0.11	1.00e+00	(-4.72,4.50)
	E([+]:[-])-E([+]:[+])	[17][3]	-1.68	7.03e-01	(-5.77,2.40)
OmpK35:TEM	E([-]:[-])-E([-]:[+])	[52][23]	0.41	9.12e-01	(-1.22,2.03)
	E([-]:[-])-E([+]:[-])	[52][12]	2.88	2.65e-03	(0.80,4.96)
	E([-]:[-])-E([+]:[+])	[52][8]	1.21	5.74e-01	(-1.25,3.68)
	E([-]:[+])-E([+]:[-])	[23][12]	2.47	3.15e-02	(0.16,4.78)
	E([-]:[+])-E([+]:[+])	[23][8]	0.80	8.60e-01	(-1.86,3.47)
	E([+]:[-])-E([+]:[+])	[12][8]	-1.67	4.58e-01	(-4.63,1.30)
OmpK35:CTX-M	E([-]:[-])-E([-]:[+])	[23][52]	0.22	9.85e-01	(-1.42,1.86)
	E([-]:[-])-E([+]:[-])	[23][8]	1.59	4.12e-01	(-1.10,4.28)
	E([-]:[-])-E([+]:[+])	[23][12]	2.67	1.82e-02	(0.34,5.00)
	E([-]:[+])-E([+]:[-])	[52][8]	1.37	4.74e-01	(-1.11,3.86)
	E([-]:[+])-E([+]:[+])	[52][12]	2.45	1.52e-02	(0.35,4.55)
	E([+]:[-])-E([+]:[+])	[8][12]	1.08	7.82e-01	(-1.91,4.06)
	E([-]:[-])-E([-]:[+])	[36][39]	-0.44	8.71e-01	(-1.96,1.07)

OmpK35:OXA	E([-]:[-])-E([+]:[-])	[36][9]	1.85	2.02e-01	(-0.59,4.30)
	E([-]:[-])-E([+]:[+])	[36][11]	1.86	1.44e-01	(-0.40,4.12)
	E([-]:[+])-E([+]:[-])	[39][9]	2.29	7.09e-02	(-0.13,4.72)
	E([-]:[+])-E([+]:[+])	[39][11]	2.30	4.16e-02	(0.06,4.54)
	E([+]:[-])-E([+]:[+])	[9][11]	0.01	1.00e+00	(-2.94,2.96)
OmpK37:SHV	E([-]:[-])-E([-]:[+])	[43][4]	0.43	9.89e-01	(-3.19,4.06)
	E([-]:[-])-E([+]:[-])	[43][43]	-0.40	8.95e-01	(-1.90,1.09)
	E([-]:[-])-E([+]:[+])	[43][5]	-0.13	1.00e+00	(-3.41,3.15)
	E([-]:[+])-E([+]:[-])	[4][43]	-0.84	9.30e-01	(-4.46,2.79)
	E([-]:[+])-E([+]:[+])	[4][5]	-0.57	9.89e-01	(-5.22,4.09)
	E([+]:[-])-E([+]:[+])	[43][5]	0.27	9.96e-01	(-3.01,3.55)
OmpK37:TEM	E([-]:[-])-E([-]:[+])	[34][13]	-0.53	9.26e-01	(-2.78,1.72)
	E([-]:[-])-E([+]:[-])	[34][30]	-0.81	6.11e-01	(-2.54,0.92)
	E([-]:[-])-E([+]:[+])	[34][18]	-0.14	9.98e-01	(-2.15,1.87)
	E([-]:[+])-E([+]:[-])	[13][30]	-0.28	9.89e-01	(-2.57,2.01)
	E([-]:[+])-E([+]:[+])	[13][18]	0.39	9.77e-01	(-2.12,2.90)
	E([+]:[-])-E([+]:[+])	[30][18]	0.67	8.28e-01	(-1.39,2.73)
OmpK37:CTX-M	E([-]:[-])-E([-]:[+])	[13][34]	0.64	8.81e-01	(-1.62,2.90)
	E([-]:[-])-E([+]:[-])	[13][18]	0.13	9.99e-01	(-2.39,2.65)
	E([-]:[-])-E([+]:[+])	[13][30]	0.00	1.00e+00	(-2.29,2.30)
	E([-]:[+])-E([+]:[-])	[34][18]	-0.51	9.11e-01	(-2.53,1.51)
	E([-]:[+])-E([+]:[+])	[34][30]	-0.63	7.73e-01	(-2.37,1.10)
	E([+]:[-])-E([+]:[+])	[18][30]	-0.12	9.99e-01	(-2.19,1.94)
OmpK37:OXA	E([-]:[-])-E([-]:[+])	[24][23]	0.01	1.00e+00	(-2.01,2.03)
	E([-]:[-])-E([+]:[-])	[24][21]	-0.09	9.99e-01	(-2.16,1.98)
	E([-]:[-])-E([+]:[+])	[24][27]	-0.65	8.15e-01	(-2.59,1.29)
	E([-]:[+])-E([+]:[-])	[23][21]	-0.10	9.99e-01	(-2.19,1.99)
	E([-]:[+])-E([+]:[+])	[23][27]	-0.66	8.13e-01	(-2.63,1.30)
	E([+]:[-])-E([+]:[+])	[21][27]	-0.56	8.84e-01	(-2.58,1.45)
SHV:TEM	E([-]:[-])-E([-]:[+])	[64][22]	-0.02	9.99e-01	(-1.58,1.54)
	E([-]:[-])-E([+]:[-])	[64][0]	0.32	9.40e-01	(-1.93,2.56)
	E([-]:[+])-E([+]:[-])	[22][0]	0.34	9.45e-01	(-2.16,2.83)
SHV:CTX-M	E([-]:[-])-E([-]:[+])	[28][58]	0.30	9.60e-01	(-1.30,1.90)
	E([-]:[-])-E([+]:[-])	[28][3]	0.56	9.85e-01	(-3.66,4.78)
	E([-]:[-])-E([+]:[+])	[28][6]	0.51	9.74e-01	(-2.62,3.63)
	E([-]:[+])-E([+]:[-])	[58][3]	0.26	9.98e-01	(-3.86,4.37)
	E([-]:[+])-E([+]:[+])	[58][6]	0.20	9.98e-01	(-2.78,3.18)
	E([+]:[-])-E([+]:[+])	[3][6]	-0.06	1.00e+00	(-4.97,4.86)
SHV:OXA	E([-]:[-])-E([-]:[+])	[41][45]	-0.44	8.69e-01	(-1.93,1.06)
	E([-]:[-])-E([+]:[-])	[41][4]	-0.43	9.90e-01	(-4.06,3.20)
	E([-]:[-])-E([+]:[+])	[41][5]	0.51	9.78e-01	(-2.77,3.79)
	E([-]:[+])-E([+]:[-])	[45][4]	0.01	1.00e+00	(-3.60,3.63)
	E([-]:[+])-E([+]:[+])	[45][5]	0.94	8.73e-01	(-2.32,4.21)
	E([+]:[-])-E([+]:[+])	[4][5]	0.93	9.53e-01	(-3.71,5.58)
TEM:CTX-M	E([-]:[-])-E([-]:[+])	[12][52]	0.20	9.95e-01	(-2.03,2.42)
	E([-]:[-])-E([+]:[-])	[12][19]	0.01	1.00e+00	(-2.55,2.57)
	E([-]:[-])-E([+]:[+])	[12][12]	0.60	9.46e-01	(-2.24,3.43)
	E([-]:[+])-E([+]:[-])	[52][19]	-0.19	9.94e-01	(-2.05,1.67)
	E([-]:[+])-E([+]:[+])	[52][12]	0.40	9.66e-01	(-1.83,2.62)
	E([+]:[-])-E([+]:[+])	[19][12]	0.59	9.32e-01	(-1.98,3.15)
TEM:OXA	E([-]:[-])-E([-]:[+])	[26][38]	-0.14	9.97e-01	(-1.91,1.62)

	E([-]:[-])-E([+]:[-])	[26][19]	0.24	9.91e-01	(-1.86,2.33)
	E([-]:[-])-E([+]:[+])	[26][12]	-0.40	9.73e-01	(-2.82,2.02)
	E([-]:[+])-E([+]:[-])	[38][19]	0.38	9.56e-01	(-1.57,2.33)
	E([-]:[+])-E([+]:[+])	[38][12]	-0.26	9.91e-01	(-2.56,2.04)
	E([+]:[-])-E([+]:[+])	[19][12]	-0.64	9.15e-01	(-3.20,1.92)
CTX-M:OXA	E([-]:[-])-E([-]:[+])	[19][12]	-1.26	5.69e-01	(-3.80,1.28)
	E([-]:[-])-E([+]:[-])	[19][26]	-0.25	9.89e-01	(-2.33,1.83)
	E([-]:[-])-E([+]:[+])	[19][38]	-0.20	9.93e-01	(-2.13,1.74)
	E([-]:[+])-E([+]:[-])	[12][26]	1.00	6.95e-01	(-1.40,3.41)
	E([-]:[+])-E([+]:[+])	[12][38]	1.06	6.19e-01	(-1.22,3.34)
	E([+]:[-])-E([+]:[+])	[26][38]	0.06	1.00e+00	(-1.70,1.81)

Table S10. ESBL and non-ESBL Single gene correlation between species and c/t resistance using PCR data

Species	Gene	[+]	[-]	mean([+])-mean([-])	p-value
All	EmrD	81	15	1.08	0.8616
	tetR	13	83	-0.89	0.1432
	OmpK35	21	75	-2.12	**0.0006**
	OmpK37	49	47	0.36	0.7475
	SHV	9	86	-0.32	0.3155
	TEM	31	64	-0.08	0.4454
	CTX-M	64	31	-0.27	0.3241
	OXA	50	45	0.31	0.7131
E. coli	EmrD	69	7	1.29	0.8160
	tetR	3	73	0.87	0.8742
	OmpK35	6	70	-1.20	0.1119
	OmpK37	37	39	0.64	0.8730
	SHV	7	69	-0.48	0.1693
	TEM	26	50	-0.10	0.4350
	CTX-M	52	24	-0.45	0.2331
	OXA	40	36	0.45	0.7874
K. pneumoniae	EmrD	10	7	-0.36	0.4217
	tetR	9	8	-0.67	0.3459
	OmpK35	13	4	-4.36	**0.0231**
	OmpK37	11	6	-0.60	0.3805
	SHV	2	15	0.19	0.5198
	TEM	4	13	-0.22	0.4500
	CTX-M	11	6	0.04	0.5103
	OXA	10	7	-0.73	0.3413

Chapter 4 supplementals

Table S11. COPUS coding scheme – instructor codes (adapted from Smith et al. 2013)

Collapsed COPUS Code	COPUS Code	COPUS Code Description
Presenting	Lecturing (Lec)	Lecturing (presenting content, deriving mathematical results, present a problem solution, etc.)
	Real-time Writing (RtW)	Realtime writing on board, doc. projector, etc. (often checked off with Lec)
	Demo or Video (D/V)	Showing or conducting a demo, experiment, simulation, video, or animation
Guiding	Follow-up (Fup)	Follow-up/feedback on clicker question or activity to entire class
	Posing a question (PQ)	Posing non-clicker question to students (nonrhetorical)
	Clicker question (CQ)	Asking a clicker question (mark the entire time the instructor is using a clicker question, not just when first asked
	Answering questions (AnQ)	Listening to and answering student questions with the entire class listening
	Moving and guiding (MG)	Moving through class guiding ongoing student work during active learning tasks
	One on one (1o1)	One on one extended discussion with one or a few individuals, not paying attention to the rest of the class (can be along with MG or AnQ)
Administering	Administration (Adm)	Administration (assign homework, return tests, etc.)
Other	Waiting (W)	Waiting when there is an opportunity for an instructor to be interacting with or observing/listening to student or group activities and the instructor is not doing so
	Other (O)	Other

Table S12. Sample COPUS coding matrix (adapted from Smith et al. 2013)

Date: _____ **Class:** _____ **Instructor:** _____

No students: _____ **Arranged how?:** _____

1. **L**- Listening; **Ind**- Individual thinking; **CG**- Clicker Q discussion; **WG**- Worksheet group work; **OG**- Other group work; **AnQ**- Answer Q; **SQ**- Student Q; **WC**- Whole class discuss; **Prd**- Predicting; **SP**- Student present; **TQ**- Test/quiz; **W**- Waiting; **O**- Other

2. **Lec**- Lecturing; **Rtw**- Writing; **FUp**- Follow-up; **PQ**- Pose Q; **CQ**- Clicker Q; **AnQ**- Answer Q; **MG**- Moving/Guiding; **1o1**- One on one; **D/V**- Demo+; **Adm**- Admin; **W**- Waiting; **O**- Other

HOW TO USE MATRIX: for each 2 min interval, check columns to show what is happening in each category. OK to check multiple columns

time (min)	1. Students doing												2. Instructor doing											**Comments**: EG: explain difficult coding choices, flag key points for feedback for the instructor, identify good analogies, etc.
	L	Ind	CG	WG	OG	AnQ	SQ	WC	Prd	SP	T/Q	W	O	Lec	RtW	FUp	PQ	CQ	AnQ	MG	1o1	D/V	Adm	W	O	
0 - 2																									
2 - 4																									
4 - 6																									
6 - 8																									
8 - 10																									

Table S13. CDOP coding scheme (adapted from Kranzfelder et al. 2019a)

Discourse Approach	CDOP Code	CDOP Code Description	Examples of classroom discourse
Authoritative, Non-Interactive	Sharing	Teacher shares information, answers students' question, or provides instructions for finding the solution	Teacher*: "Just think of, kind of, chromatid pairs, sister chromatid paired, it's a little easier to think of the numbers."*
	Real-worlding	Teacher relates idea to conventional knowledge, broader perspective, and instructors or students personally	Teacher: *"Successful genotypes- look around the room. Nothing but winners in this room, right? We have all made it to reproductive age."*
	Linking	Teacher associates past topic to current topic	Student: *"You don't have a bigger potential as well because there's more connections, there's more access to the axon terminals?"* Teacher: *"Well, remember, we had that summation of action potentials. We had an action potential and we had the nodes and it could split off."*
	Forecasting	Teacher associates current topic to future topic	Teacher: *"You're going to do something in lab actually focused on human population and population growth."*
Authoritative, Interactive	Evaluating	Teacher repeats, accepts and/or rejects students' response, or acknowledges that they don't know the answer to a student's question	Student: *"And then in the first case, it would be once chance times one chance which is still one sixteenth."* Teacher: *"Right."*
	Generative	Teacher asks student to recall facts, and basic concepts, or related information	Teacher: *"Those come together in fertilization to make a zygote, right?"* Student: *"Yes."*
	Checking-in	Teacher asks student to recall facts, basic concepts, or related information	Teacher: *"Does that make sense?; Do you have any questions?; How's it going?; Are we good?"*
Dialogic, Interactive	Clarifying	Teacher asks student to elaborate on condensed, cryptic, or inexplicit statement	Teacher: *"Can you say more about that? What do you mean by that? Can you give an example?"*
	Connecting	Teacher asks student to associate past topic to current topic	Teacher: *"Costs of sex that haven't been mentioned plus what we've been talking about for the last week."* Student: *"Is it overpopulation?"*
	Contextualizing	Teacher asks students to relate idea to conventional knowledge, broader perspective, and their personal experiences	Teacher: *"Anyone have an example that they really want to hear about/talk about (referring to student responses to finding analogies between cell processes and common household items)?"*
	Representing	Teacher asks student to create a visual or mathematical representation of content	Teacher: *"Think about how you could draw that out, too."*
	Constructing	Teacher asks students to build knowledge by interpreting and/or making judgments based on evidence, data, and/or model	Teacher: *"In your own words, what is your conclusion when you look at those data?"*
	Requesting	Teacher asks student to justify or explain their reasoning	Teacher: *"I'm liking what I see but explain it to me"* (referring to student whiteboard work

			calculating the number of fertilization events that produce a specific offspring).
	Explaining	Teacher asks student to explain reasoning to other students	Teacher: *"Can you explain your work to everybody else at your table so that they can Table that out?"*
	Challenging	Teacher asks student to evaluate another student's idea	Teacher: *"Cost of sex?"* Student: *"Pregnancy."* Teacher: *"I acknowledge that it's a good point, and why is there a problem with calling pregnancy a cost evolutionarily?"*
Other	No content discourse	Teacher is not talking or asking students to talk about content (see examples of *Instructor Talk* in Seidel et al. (2015))	
	Other	TDM not described by these codes	

Table S14. CDOP coding matrix (adapted from Kranzfelder et al. 2019a)

Instructor: _____ **Observer:** _____ **Course:** _____

Class date: _____ **Class size:** _____ **Class layout:** _____

1. **Share**-Sharing; **Realw**-Real-worlding; **Frcst**-Forecasting; **Link**-Linking;

2. **Eval**-Evaluating; **Gener**-Generative; **Check**-Checking;

3. **Clari**-Clarifying; **Conn**-Connecting; **Cntex**-Contextualizing; **Repre**-Representing; **Const**-Constructing; **Reqst**-Requesting; **Expl**-Explaining; **Chall**-Challenging

4. **Other**-Other; **NCD**-No content discourse

HOW TO USE MATRIX: Put a check under all codes that happen anytime in each 2-minute time period. If no codes fit, choose "Other" and explain in notes.

Check multiple codes where appropriate, except NCD is only checked when no other codes are present in that period. Clarify code choices with comments in notes.

time (min)	1. Authoritative, Non-Interactive				2. Authoritative, Interactive			3. Dialogic, Interactive								4. Other		Notes
	Share	RealW	Link	Frcst	Eval	Gener	Check	Clari	Conn	Cntex	Repre	Const	Reqst	Expl	Chall	Other	NCD	
0 - 2																		
2 - 4																		
4 - 6																		
6 - 8																		
8 - 10																		

Table S15. COPUS training - inter-rater reliability calculations among coders

Instructor Code	Class Session	Coder pairs	No of minutes	Fleiss' Kappa	Confidence Intervals	
					Lower	Upper
144	4-Nov-19	All 16 coders	30	0.55	0.55	0.56

Table S16. CDOP training - inter-rater reliability calculations among coder pairs

Instructor	Class	Coders	No. of minutes	Cohen's Kappa	SE	Confidence Intervals	
ID	**Session**					**Lower**	**Upper**
148	10-Feb-2019	Coder 1/Coder 2	30	0.81	0.052	0.71	0.91
129	10-Mar-2020	Coder 1-3	30	0.83	NA	0.76	0.90
129	10-Mar-2020	Coder 1-4	30	0.74	NA	0.69	0.79

Table S17. CDOP Inter-rater reliability calculations among coder pairs for 27% of the audio files

Instructor ID	Class Session	Coder pairs (1st coder/2nd coder)	No. of minutes	Cohen's Kappa	SE	Confidence Intervals	
						Lower	Upper
121	4-Feb-2020	Coder 1/Coder 2	75	0.79	0.042	0.71	0.87
122	9-Mar-2020	Coder 1/Coder 2	75	0.62	0.044	0.53	0.71
125	7-Feb-2020	Coder 1/Coder 2	50	0.89	0.041	0.81	0.97
126	10-Feb-2020	Coder 1/Coder 2	52	0.86	0.034	0.79	0.92
128	5-Feb-2020	Coder 1/Coder 2	76	0.78	0.033	0.72	0.85
131	7-Apr-2020	Coder 3/ Coder 2	50	0.85	0.047	0.75	0.94
134	11-Feb-2020	Coder 1/Coder 2	62	0.86	0.043	0.77	0.94
136	5-Feb-2020	Coder 1/Coder 2	71	0.93	0.025	0.88	0.98
137	6-Nov-2019	Coder 1/Coder 2	38	0.79	0.056	0.68	0.9
138	6-Apr-2020	Coder 1/Coder 2	83	0.79	0.042	0.71	0.87
141	3-Mar-2020	Coder 1/Coder 2	73	0.61	0.04	0.53	0.7
143	18-Feb-2020	Coder 3/Coder 1	69	0.98	0.012	0.95	1
145	2-Dec-2019	Coder 3/Coder 1	53	0.89	0.048	0.71	0.9
145	4-May-2020	Coder 3/Coder 1	65	0.85	0.047	0.76	0.94
147	13-Sep-2019	Coder 3/Coder 1	50	0.78	0.57	0.66	0.89
148	2-Dec-2019	Coder 3/Coder 1	73	0.98	0.011	0.96	1
149	7-Oct-2018	Coder 1/Coder 2	52	0.75	0.06	0.63	0.86
150	19-Apr-2019	Coder 4/Coder 1	44	0.64	0.072	0.5	0.78
151	22-Feb-2019	Coder 3/Coder 1	41	0.87	0.32	0.8	0.93
152	22-Mar-2019	Coder 4/Coder 1	50	0.75	0.045	0.66	0.84
153	27-Feb-2020	Coder 3/Coder 1	52	0.9	0.032	0.84	0.97

154	29-Oct-2018	Coder 1/Coder 2	50	0.95	0.021	0.91	0.99
155	6-Dec-2019	Coder 4/Coder 1	49	0.94	0.034	0.88	1.01
157	7-Apr-2020	Coder 1/Coder 2	70	0.76	0.051	0.66	0.86
158	7-Apr-2020	Coder 1/Coder 2	70	0.97	0.018	0.94	1.01
		Average 0.83					

Table S18. Pairwise comparisons using paired Wilcoxon signed-rank test collapsed COPUS codes

COPUS codes 1	COPUS codes 2	*n*	W-Statistic	P	P adjusted	Significance levels
Presenting	Guiding	74	2042	<0.001	0.003	**
Presenting	Administering	74	2775	<0.001	<0.001	****
Presenting	Other	74	2775	<0.001	<0.001	****
Guiding	Administering	74	2701	<0.001	<0.001	****
Guiding	Other	74	2701	<0.001	<0.001	****
Administering	Other	74	1697	<0.001	<0.001	****

n = number of class sessions
W-Statistic approximates a normal distribution. Higher numbers mean we reject the null hypothesis
P = lower p-values help us reject the null hypothesis (interactions are not due to chance)
P adjusted = adjustments in p-value to confirm rejection of the null hypothesis
Significance level = more stars indicate higher significance

Table S19. Percentage of instructor COPUS codes across all class sessions

COPUS codes	*n*	Mean (%)	SD (%)	Median (%)	Min (%)	Max (%)	IQR (%)
Individual							
Lecturing	74	37.4	15.3	37.9	4.3	79	18
Posing Questions	74	18.1	11.6	16.7	0.0	46.2	15.5
Real-time Writing	74	15.4	13	17.7	0.0	39.1	27.6
Moving and Guiding	74	6.4	9.6	0.0	0.0	41.9	9
Answering Questions	74	5.7	4.6	5	0.0	20.5	6.5
Follow-up	74	5.1	7.2	1.8	0.0	34.3	7.7
Administration	74	4.4	4.7	3.4	0.0	24	4.1
Clicker Question	74	4.3	6.8	0.0	0.0	32.9	6.8
Demo/Video	74	2.1	4.2	0.0	0.0	19.3	2.3
Other	74	1.2	2.3	0.0	0.0	12.5	1.7
One-on-One	74	0.3	1.1	0.0	0.0	5.6	0.0
Waiting	74	0.3	1	0.0	0.0	6.4	0.0
Collapsed							
Presenting	74	54.8	18.3	56.5	11	100	24.4
Guiding	74	39.6	17.1	38.2	0.0	87.3	22.6
Administering	74	4.4	4.7	3.4	0.0	24	4.1
Other	74	1.4	2.5	0.0	0.0	12.5	2.1

For individual code explanations refer to Table S1
n = number of class sessions
SD = standard deviation
IQR = interquartile range
Min = minimum
Max = maximum

Table S20. Percentage of CDOP codes across all class sessions

CDOP codes	*n*	Mean (%)	SD (%)	Median (%)	Min (%)	Max (%)	IQR (%)
Individual							
Sharing	74	43.1	14.6	37.7	20.7	20.3	85.8
Generative	74	16.2	7.6	18.2	11.1	0.0	27.7
Evaluating	74	12.8	6.5	13.9	9.6	0.0	24.5
Checking-in	74	7.5	5.3	7.7	7.5	0.0	17.5
Real-worlding	74	5.9	6.9	3.3	7.0	0.0	33
Non-content discourse	74	3.7	4.6	2.3	5.1	0.0	21.8
Linking	74	2.3	2.5	1.7	3.5	0.0	9.3
Clarifying	74	2.2	2.6	1.7	3.7	0.0	9.6
Requesting	74	1.5	2.2	0.0	2.7	0.0	8.6
Forecasting	74	1.4	1.9	1.0	2.0	0.0	10.8
Explaining	74	1.3	2.4	0.0	1.7	0.0	10.7
Constructing	74	0.8	2.0	0.0	0.0	0.0	8.7
Representing	74	0.7	1.2	0.0	1.1	0.0	4.7
Challenging	74	0.5	1.2	0.0	0.0	0.0	5
Contextualizing	74	0.5	1.2	0.0	0.0	0.0	5.6
Connecting	74	0.4	1.1	0.0	0.0	0.0	5.4
Other	74	0.0	0.0	0.0	0.0	0.0	1.0
Collapsed							
Authoritative, Non-Interactive	74	52.6	16.7	47.5	22.2	29	100
Authoritative, Interactive	74	36.4	14.2	39.7	18.2	0.0	61.6
Dialogic, Interactive	74	7.5	6.4	7.2	9.8	0.0	24.5
Other	74	3.7	4.6	2.3	5.1	0.0	21.8

For individual code explanations refer to Table S3.
n = number of class sessions
SD = standard deviation
IQR = interquartile range
Min = minimum
Max = maximum

Table S21 Pairwise comparisons using paired Wilcoxon signed-rank test for collapsed CDOP codes

CDOP code 1	CDOP code 2	*n*	W-Statistic	P	P adj	Significance
Authoritative, Non-Interactive	Authoritative, Interactive	74	1940	<0.001	0.003	**
Authoritative, Non-Interactive	Dialogic, Interactive	74	2775	<0.001	<0.001	****
Authoritative, Non-Interactive	Other	74	2775	<0.001	<0.001	****
Authoritative, Interactive	Dialogic, Interactive	74	2627	<0.001	<0.001	****
Authoritative, Interactive	Other	74	2555	<0.001	<0.001	****
Dialogic, Interactive	Other	74	1713.5	<0.001	0.002	**

n = number of class sessions
W-Statistic approximates a normal distribution. Higher numbers mean we reject the null hypothesis
P = lower p-values help us reject the null hypothesis (interactions are not due to chance)
P adjusted = adjustments in p-value to confirm rejection of the null hypothesis
Significance level = more stars indicate higher significance

Table S22. COPUS/CDOP Correlation P-values

COPUS/CDOP codes	Presenting	Guiding	Administering	Other	Authoritative, Non-Interactive	Authoritative, Interactive	Dialogic, Interactive	Other
Presenting	0.0	<0.001	0.7	1.0	<0.001	0.02	<0.001	1.0
Guiding	<0.001	0.0	1.0	1.0	<0.001	<0.01	<0.001	1.0
Administering	0.02	0.7	0.0	1.0	1.0	1.0	1.0	0.1
Other	0.1	0.9	0.5	0.0	1.0	1.0	1.0	0.06
Authoritative, Non-Interactive	<0.001	<0.001	0.6	0.9	0.0	<0.001	<0.001	1.0
Authoritative, Interactive	<0.001	<0.001	0.6	0.3	<0.001	0.0	1.0	0.06
Dialogic, Interactive	<0.001	<0.001	0.1	0.9	<0.001	0.04	0.0	1.0
Other	0.1	0.6	<0.01	0.002	0.8	<0.01	0.9	0.0

Table S23. COPUS/CDOP Correlation R-values

COPUS/CDOP codes	Presenting	Guiding	Administering	Other	Authoritative, Non-Interactive	Authoritative, Interactive	Dialogic, Interactive	Other
Presenting	1	-0.9	-0.3	-0.2	0.64	-0.38	-0.64	-0.2
Guiding	-0.9	1	0.05	0.01	-0.67	0.45	0.66	0.05
Administering	-0.3	0.05	1	0.08	-0.2	-0.06	0.2	0.3
Other	-0.2	0.01	0.08	1	-0.02	-0.1	-0.008	0.4

Table S24. Percentage of collapsed COPUS codes across STEM discipline

STEM discipline	COPUS codes	*n*	Mean (%)	SD (%)	Median (%)	IQR (%)	Min (%)	Max (%)
biology	Presenting	30	47.1	19.46	44.82	30.68	10.91	82.05
biology	Guiding	30	46.29	18.42	48.24	18.78	15.79	87.27
biology	Administering	30	4.98	5.77	3.57	5.15	0.0	23.91
biology	Other	30	1.62	2.49	0.0	2.56	0.0	9.68
chemistry	Presenting	22	65.9	15.35	67.14	20.31	40.68	100
chemistry	Guiding	22	30.7	15.4	32.58	20.31	0.0	56.52
chemistry	Administering	22	2.42	2.39	2.19	3.83	0.0	8.51
chemistry	Other	22	0.98	1.27	0.0	2.04	0.0	3.45
mathematics	Presenting	10	58.14	14.09	62.3	19	33.68	73.91
mathematics	Guiding	10	37.87	13.35	35.88	19.07	23.08	65.26
mathematics	Administering	10	3.09	1.96	2.78	1.93	1.05	7.69
mathematics	Other	10	0.9	1.94	0.0	1.01	0.0	6.15
other STEM	Presenting	12	50.52	12.55	50.13	13.92	30.56	75.32
other STEM	Guiding	12	40.47	12.47	40.06	20.67	20.78	61.11
other STEM	Administering	12	7.08	4.54	5.66	3.55	3.19	18.75
other STEM	Other	12	1.93	3.92	0.0	1.28	0.0	12.5

For collapsed code explanations refer to Table S1.
n = number of class sessions
SD = standard deviation
IQR = interquartile range
Min = minimum
Max = maximum

Table S25. Comparison of STEM disciplines and collapsed COPUS codes by two-way ANOVA and Post-Hoc comparison

STEM Disciplines x Collapsed COPUS Codes	estimate	SE	DF	T ratio	P value
biology Presenting - chemistry Presenting	**-95.21**	**22.88**	**280**	**-4.16**	**<0.01**
biology Presenting - mathematics Presenting	**-66.25**	**29.76**	**280**	**-2.23**	**0.03**
biology Presenting - other STEM Presenting	0.45	27.84	280	0.02	0.99
chemistry Presenting - mathematics Presenting	28.96	31.08	280	0.93	0.35
chemistry Presenting - other STEM Presenting	**95.66**	**29.25**	**280**	**3.27**	**<0.01**
mathematics Presenting - other STEM Presenting	66.7	34.9	280	1.91	0.06
biology Guiding - chemistry Guiding	**83.23**	**22.88**	**280**	**3.64**	**<0.01**
biology Guiding - mathematics Guiding	52.35	29.76	280	1.76	0.08
biology Guiding - other STEM Guiding	23.93	27.84	280	0.86	0.39
chemistry Guiding - mathematics Guiding	-30.88	31.08	280	-0.99	0.32
chemistry Guiding - other STEM Guiding	**-59.31**	**29.25**	**280**	**-2.03**	**0.04**
mathematics Guiding - other STEM Guiding	-28.43	34.9	280	-0.81	0.42
biology Administering - chemistry Administering	31.28	22.88	280	1.37	0.17
biology Administering - mathematics Administering	24.25	29.76	280	0.81	0.42
biology Administering - other STEM Administering	-47.19	27.84	280	-1.7	0.09
chemistry Administering - mathematics Administering	-7.03	31.08	280	-0.23	0.82
chemistry Administering - other STEM Administering	-78.47	29.25	280	-2.68	0.01
mathematics Administering - other STEM Administering	-71.44	34.9	280	-2.05	0.04
biology Other - chemistry Other	6.01	22.88	280	0.26	0.79
biology Other - mathematics Other	27.93	29.76	280	0.94	0.35
biology Other - other STEM Other	22.11	27.84	280	0.79	0.43
chemistry Other - mathematics Other	21.93	31.08	280	0.71	0.48
chemistry Other - other STEM Other	16.1	29.25	280	0.55	0.58
mathematics Other - other STEM Other	-5.83	34.9	280	-0.17	0.87

Bold = significant interactions described in results.

Table S26. Percentage of collapsed CDOP codes across STEM disciplines

STEM disciplines	CDOP codes	*n*	Mean (%)	SD (%)	Median (%)	IQR (%)	Min (%)	Max (%)
biology	Authoritative, Non-Interactive	30	51.01	14.48	47.41	19.37	28.99	83.72
biology	Authoritative, Interactive	30	36.24	12.67	36.7	13.07	11.11	61.54
biology	Dialogic, Interactive	30	7.37	5.68	7.9	9.31	0.0	20.83
biology	Other	30	5.38	5.47	4.54	6.03	0.0	21.74
chemistry	Authoritative, Non-Interactive	22	60.71	18.81	55.29	32.32	36.07	100
chemistry	Authoritative, Interactive	22	32.29	16.8	38.53	30.59	0.0	52.05
chemistry	Dialogic, Interactive	22	4.72	6.02	1.74	9.22	0.0	16.92
chemistry	Other	22	2.28	3.42	0.0	3.1	0.0	11.11
mathematics	Authoritative, Non-Interactive	10	49.57	19.23	43.3	25.43	30.53	90.91
mathematics	Authoritative, Interactive	10	37.71	16.31	42.66	18.44	2.27	57.14
mathematics	Dialogic, Interactive	10	10	8.42	8.74	13.27	0.0	24.43
mathematics	Other	10	2.71	2.13	2.18	2.83	0.0	6.78
other STEM	Authoritative, Non-Interactive	12	43.72	8.78	42.01	3.92	32.98	66.15
other STEM	Authoritative, Interactive	12	43.03	7.57	42.75	8.3	26.15	55.32
other STEM	Dialogic, Interactive	12	10.42	5.37	9.55	5.15	2.99	21.55
other STEM	Other	12	2.83	4.2	1.13	3.47	0.0	13.89

For collapsed code explanations refer to Table S3.
n = number of class sessions
SD = standard deviation
IQR = interquartile range
Min = minimum
Max = maximum

Table S27. Comparison of STEM disciplines and collapsed CDOP codes by two-way ANOVA and Post- Hoc comparison

STEM Disciplines x Collapsed CDOP Codes	Estimate	SE	DF	T-ratio	P -value
biology Authoritative, Non-Interactive - chemistry Authoritative, Non-Interactive	**-48.28**	**23.21**	**280**	**-2.08**	**0.04**
biology Authoritative, Non-Interactive - mathematics Authoritative, Non-Interactive	11.83	30.19	280	0.39	0.7
biology Authoritative, Non-Interactive - other STEM Authoritative, Non-Interactive	**59.37**	**28.24**	**280**	**2.1**	**0.04**
chemistry Authoritative, Non-Interactive - mathematics Authoritative, Non-Interactive	60.12	31.53	280	1.91	0.06
chemistry Authoritative, Non-Interactive - other STEM Authoritative, Non-Interactive	**107.65**	**29.67**	**280**	**3.63**	**<0.01**
mathematics Authoritative, Non-Interactive - other STEM Authoritative, Non-Interactive	47.53	35.4	280	1.34	0.18
biology Authoritative, Interactive - chemistry Authoritative, Interactive	10.13	23.21	280	0.44	0.66
biology Authoritative, Interactive - mathematics Authoritative, Interactive	-16.3	30.19	280	-0.54	0.59
biology Authoritative, Interactive - other STEM Authoritative, Interactive	**-62.02**	**28.24**	**280**	**-2.2**	**0.03**
chemistry Authoritative, Interactive - mathematics Authoritative, Interactive	-26.43	31.53	280	-0.84	0.4
chemistry Authoritative, Interactive - other STEM Authoritative, Interactive	-72.14	29.67	280	-2.43	0.02
mathematics Authoritative, Interactive - other STEM Authoritative, Interactive	-45.72	35.4	280	-1.29	0.2
biology Dialogic, Interactive - chemistry Dialogic, Interactive	35.23	23.21	280	1.52	0.13
biology Dialogic, Interactive - mathematics Dialogic, Interactive	-19.35	30.19	280	-0.64	0.52
biology Dialogic, Interactive - other STEM Dialogic, Interactive	-34.7	28.24	280	-1.23	0.22
chemistry Dialogic, Interactive - mathematics Dialogic, Interactive	-54.58	31.53	280	-1.73	0.08
chemistry Dialogic, Interactive - other STEM Dialogic, Interactive	**-69.93**	**29.67**	**280**	**-2.36**	**0.02**
mathematics Dialogic, Interactive - other STEM Dialogic, Interactive	-15.35	35.4	280	-0.43	0.66
biology Other - chemistry Other	37.69	23.21	280	1.62	0.11
biology Other - mathematics Other	24.83	30.19	280	0.82	0.41
chemistry Other - mathematics Other	-12.86	31.53	280	-0.41	0.68
chemistry Other - other STEM Other	-6.24	29.67	280	-0.21	0.83
mathematics Other - other STEM Other	6.62	35.4	280	0.19	0.85

Bold = Significant interactions described in results.

Table S28. Percentage of collapsed COPUS codes across instructor types

Instructor Types	COPUS Codes	*n*	Mean (%)	SD (%)	Median (%)	IQR (%)	Min (%)	Max (%)
Lecturers	Presenting	32	59.56	17.84	60.43	28.29	28.17	100
Lecturers	Guiding	32	34.36	16.3	34.66	23.62	0.0	70.42
Lecturers	Administering	32	4.3	3.94	3.45	4.06	0.0	18.75
Lecturers	Other	32	1.78	3.04	0.0	2.67	0.0	12.5
Research	Presenting	27	54.1	17.79	53.47	24.63	15.38	82.05
Research	Guiding	27	41.81	17.77	43.56	25.31	14.89	84.62
Research	Administering	27	3.06	2.69	2.97	4.56	0.0	8.57
Research	Other	27	1.02	1.66	0.0	1.81	0.0	6.82
Teaching	Presenting	15	45.59	17.26	47.69	29.19	10.91	69.05
Teaching	Guiding	15	46.68	14.77	44.68	14.65	28.57	87.27
Teaching	Administering	15	6.54	7.38	3.45	4.25	0.0	23.91
Teaching	Other	15	1.2	2.05	0.0	1.74	0.0	6.38

n = number of class sessions
SD = standard deviation
IQR = interquartile range
Min = minimum
Max = maximum

Table S29. Comparison of instructor types and collapsed COPUS codes by two-way ANOVA and Post-Hoc comparison

Instructor Types x Collapsed COPUS Code	Estimate	SE	Df	T-ratio	P-value
Lecturers Presenting - Research Presenting	24.08	22.18	284	1.09	0.28
Lecturers Presenting - Teaching Presenting	**59.9**	**26.56**	**284**	**2.26**	**0.02**
Research Presenting - Teaching Presenting	35.81	27.33	284	1.31	0.19
Lecturers Guiding - Research Guiding	**-43.14**	**22.18**	**284**	**-1.95**	**0.05**
Lecturers Guiding - Teaching Guiding	**-69.47**	**26.56**	**284**	**-2.62**	**0.01**
Lecturers Administering - Research Administering	13.22	22.18	284	0.6	0.55
Lecturers Administering - Teaching Administering	-16.26	26.56	284	-0.61	0.54
Research Administering - Teaching Administering	-29.49	27.33	284	-1.08	0.28
Lecturers Other - Research Other	6.76	22.18	284	0.3	0.76
Lecturers Other - Teaching Other	21.7	26.56	284	0.82	0.41
Research Other - Teaching Other	14.94	27.33	284	0.55	0.58

Bold = Significant interactions described in results.

Table S30. Percentage of collapsed CDOP codes across instructor types

Instructor types	CDOP codes	*n*	Mean (%)	SD (%)	Median (%)	IQR (%)	Min (%)	Max (%)
Research	Authoritative, Non-Interactive	27	51.54	15.96	46.97	23.4	28.99	81.01
Research	Authoritative, Interactive	27	37.24	12.86	38.46	17.37	13.51	57.14
Research	Dialogic, Interactive	27	7.78	4.91	8.57	6.43	0.0	16.92
Research	Other	27	3.44	4.92	1.89	4.77	0.0	21.74
Teaching	Authoritative, Non-Interactive	15	41.74	6.89	41.25	6.77	30.53	55.26
Teaching	Authoritative, Interactive	15	42.88	6.99	42.5	6.71	24.24	55.32
Teaching	Dialogic, Interactive	15	11.17	6.69	11.54	6.77	0.0	24.43
Teaching	Other	15	4.2	5.07	2.13	7.6	0.0	16.36
Lecturers	Authoritative, Non-Interactive	32	58.39	18.04	52.29	25.26	34.35	100
Lecturers	Authoritative, Interactive	32	32.57	16.49	36.47	23.85	0.0	61.54
Lecturers	Dialogic, Interactive	32	5.39	6.69	2.01	9.5	0.0	21.55
Lecturers	Other	32	3.65	4.02	2.44	6.37	0.0	13.89

n = number of class sessions
SD = standard deviation
IQR = interquartile range
Min = minimum
Max = maximum

Table S31. Comparison of instructor types and collapsed CDOP codes by two-way ANOVA and Post-Hoc comparison

Instructor types x collapsed CDOP codes	Estimate	SE	DF	T-ratio	P-value
Research Authoritative, Non-Interactive - Teaching Authoritative, Non-Interactive	**72.62**	**26.27**	**284**	**2.76**	**0.01**
Research Authoritative, Non-Interactive - Lecturers Authoritative, Non-Interactive	-31.98	21.32	284	-1.5	0.13
Teaching Authoritative, Non-Interactive - Lecturers Authoritative, Non-Interactive	**-104.6**	**25.53**	**284**	**-4.1**	**<0.01**
Research Authoritative, Interactive - Teaching Authoritative, Interactive	**-56.49**	**26.27**	**284**	**-2.15**	**0.03**
Research Authoritative, Interactive - Lecturers Authoritative, Interactive	24.85	21.32	284	1.17	0.24
Teaching Authoritative, Interactive - Lecturers Authoritative, Interactive	**81.34**	**25.53**	**284**	**3.19**	**<0.01**
Research Dialogic, Interactive - Teaching Dialogic, Interactive	-32.67	26.27	284	-1.24	0.21
Research Dialogic, Interactive - Lecturers Dialogic, Interactive	34.26	21.32	284	1.61	0.11
Teaching Dialogic, Interactive - Lecturers Dialogic, Interactive	**66.93**	**25.53**	**284**	**2.62**	**0.01**
Research Other - Teaching Other	-9.68	26.27	284	-0.37	0.71
Research Other - Lecturers Other	-1.87	21.32	284	-0.09	0.93
Teaching Other - Lecturers Other	7.81	25.53	284	0.31	0.76

Bold = Significant interactions described in results.

Table S32. Percentage of collapsed COPUS codes across years of faculty teaching experience at institution

Years of teaching experience	COPUS codes	*n*	Mean	SD	Median	IQR	Min	Max
0 to 5	Presenting	34	57.62	16.76	56.32	20.01	27.66	100
0 to 5	Guiding	34	35.68	14.36	35.95	17.14	0.0	61.11
0 to 5	Administering	34	5.07	4.91	3.72	3.18	0.0	21.28
0 to 5	Other	34	1.63	2.63	0.0	2.56	0.0	12.5
6 to 10	Presenting	18	54.81	19.09	53.22	32.94	26.09	85.29
6 to 10	Guiding	18	38.62	16.7	39.13	28.98	11.76	65.91
6 to 10	Administering	18	4.71	5.57	3.26	5.34	0.0	23.91
6 to 10	Other	18	1.86	3.03	0.0	2.02	0.0	9.68
over 11	Presenting	22	50.21	19.61	57.55	28.32	10.91	75.76
over 11	Guiding	22	46.37	19.73	38.16	27.05	21.15	87.27
over 11	Administering	22	2.8	2.68	2.35	4.9	0.0	8.57
over 11	Other	22	0.62	1.08	0.0	1.01	0.0	2.86

n = number of class sessions
SD = standard deviation
IQR = interquartile range
Min = minimum
Max = maximum

Table S33. Comparison of years of teaching experience and COPUS codes by two-way ANOVA and Post-Hoc comparison

Years of teaching experience x collapsed COPUS codes	Estimate	SE	DF	T-ratio	P-value
0 to 5 Presenting - 6 to 10 Presenting	13.29	25.13	284	0.53	0.6
0 to 5 Presenting - over 11 Presenting	12.81	23.59	284	0.54	0.59
6 to 10 Presenting - over 11 Presenting	-0.47	27.4	284	-0.02	0.99
0 to 5 Guiding - 6 to 10 Guiding	-24.05	25.13	284	-0.96	0.34
6 to 10 Guiding - over 11 Guiding	-6.48	27.4	284	-0.24	0.81
0 to 5 Administering - 6 to 10 Administering	12.8	25.13	284	0.51	0.61
0 to 5 Administering - over 11 Administering	35.53	23.59	284	1.51	0.13
6 to 10 Administering - over 11 Administering	22.74	27.4	284	0.83	0.41
0 to 5 Other - 6 to 10 Other	-0.01	25.13	284	0	1
0 to 5 Other - over 11 Other	15	23.59	284	0.64	0.53
6 to 10 Other - over 11 Other	15.01	27.4	284	0.55	0.58

Table S34. Percentage of collapsed CDOP codes Across years of faculty teaching experience

Years of teaching experience	Code	*n*	Mean (%)	SD(%)	Median (%)	IQR (%)	Min (%)	Max (%)
0 to 5	Authoritative, Non-Interactive	34	53.38	17.94	44.34	23.24	32.41	100
0 to 5	Authoritative, Interactive	34	37.06	15.24	40.38	21.65	0.0	61.54
0 to 5	Dialogic, Interactive	34	6.41	6.18	4.97	11.43	0.0	21.55
0 to 5	Other	34	3.15	3.91	2.01	4.49	0.0	13.89
6 to 10	Authoritative, Non-Interactive	18	55.37	17.18	50.42	21.52	34.35	90.91
6 to 10	Authoritative, Interactive	18	31.71	14.44	36.62	24.13	2.27	47.06
6 to 10	Dialogic, Interactive	18	8.32	6.59	6.88	11.08	0.0	18.64
6 to 10	Other	18	4.6	4.35	4.59	5.15	0.0	16.36
over 11	Authoritative, Non-Interactive	22	48.85	14.05	46.95	14.27	28.99	80
over 11	Authoritative, Interactive	22	39.1	11.44	40.06	13.72	13.51	57.14
over 11	Dialogic, Interactive	22	8.29	6.62	8.63	8.1	0.0	24.43
over 11	Other	22	3.76	5.54	1.59	5.22	0.0	21.74

n= number of class sessions
SD = standard deviation
IQR = interquartile range
Min = minimum
Max = maximum

Table 35. Comparison of years of teaching experience and collapsed CDOP codes by two-way ANOVA and Post-Hoc comparison

Years of teaching experience x collapsed CDOP codes	Estimate	SE	DF	T-ratio	P-value
0 - 5 Authoritative, Non-Interactive - 6 - 10 Authoritative, Non-Interactive	-17.29	24.89	284	-0.69	0.49
0 - 5 Authoritative, Non-Interactive - over 11 Authoritative, Non-Interactive	23.1	23.36	284	0.99	0.32
6 - 10 Authoritative, Non-Interactive - over 11 Authoritative, Non-Interactive	40.38	27.14	284	1.49	0.14
0 - 5 Authoritative, Interactive - 6 - 10 Authoritative, Interactive	29.86	24.89	284	1.2	0.23
0 - 5 Authoritative, Interactive - over 11 Authoritative, Interactive	-15.68	23.36	284	-0.67	0.5
6 - 10 Authoritative, Interactive - over 11 Authoritative, Interactive	-45.54	27.14	284	-1.68	0.09
0 - 5 Dialogic, Interactive - 6 - 10 Dialogic, Interactive	-19.09	24.89	284	-0.77	0.44
0 - 5 Dialogic, Interactive - over 11 Dialogic, Interactive	-18.74	23.36	284	-0.8	0.42
6 - 10 Dialogic, Interactive - over 11 Dialogic, Interactive	0.35	27.14	284	0.01	0.99
0 - 5 Other - 6 - 10 Other	-20.15	24.89	284	-0.81	0.42
0 - 5 Other - over 11 Other	-6.48	23.36	284	-0.28	0.78
6 - 10 Other - over 11 Other	13.67	27.14	284	0.5	0.61

Table S36. Percentage of collapsed COPUS codes across class size

Class size	Code	*n*	Mean (%)	SD (%)	Median (%)	IQR (%)	Min (%)	Max (%)
large	Presenting	43	54.65	18.53	57.14	25.3	20.9	100
large	Guiding	43	39.14	16.41	38.71	21.37	0.0	71.64
large	Administering	43	4.73	5.16	3.33	4.38	0.0	23.91
large	Other	43	1.48	2.31	0.0	2.11	0.0	9.68
small & medium	Presenting	31	54.85	18.13	55.77	25.98	10.91	82.05
small & medium	Guiding	31	40.18	18.19	37.86	24.22	15.79	87.27
small & medium	Administering	31	3.72	3.7	3.45	3.21	0.0	18.75
small & medium	Other	31	1.25	2.6	0.0	1.66	0.0	12.5

Small = 1-60 students
Medium = >60 ≤ 100
Large = >100 students
n = number of class sessions
SD = standard deviation
IQR = interquartile range
min = minimum; max = maximum

Table S37. Comparison of class size and collapsed COPUS codes by two-way ANOVA and Post-Hoc comparison

Class size x collapsed COPUS codes	Estimate	SE	DF	T-ratio	P-value
large Presenting - small & medium Presenting	-4.01	20.37	288	-0.2	0.84
large Guiding - small & medium Guiding	2.88	20.37	288	0.14	0.89
large Administering - small & medium Administering	6.69	20.37	288	0.33	0.74
large Other - small & medium Other	-8.27	20.37	288	-0.41	0.68

Table S38. Percentage of collapsed CDOP codes across class size

Class size	CDOP codes	*n*	Mean (%)	SD (%)	Median (%)	IQR (%)	Min (%)	Max (%)
large	Authoritative, Non-Interactive	43	52.66	17.01	46.97	21.56	28.99	100
large	Authoritative, Interactive	43	35.45	14.87	38.46	18.37	0	61.54
large	Dialogic, Interactive	43	7.38	5.91	8.11	9.64	0	21.55
large	Other	43	4.5	5.5	2.56	7.42	0	21.74
small & medium	Authoritative, Non-Interactive	31	52.32	16.43	47.89	22.89	30.53	90.91
small & medium	Authoritative, Interactive	31	37.63	13.11	41.79	16.81	2.27	53.7
small & medium	Dialogic, Interactive	31	7.51	7.11	5.32	9.56	0	24.43
small & medium	Other	31	2.54	2.27	2.13	4.17	0	7.5

n = number of class sessions
SD = standard deviation
IQR = interquartile range
min = minimum
max = maximum

Table S39. Comparison of class size and collapsed CDOP codes by two-way ANOVA and Post-Hoc comparison

Class size x collapsed COPUS codes	Estimate	SE	DF	T-ratio	P-value
small & medium Authoritative, Non-Interactive - large Authoritative, Non-Interactive	-1.36	20.16	288	-0.07	0.95
small & medium Authoritative, Interactive - large Authoritative, Interactive	16.59	20.16	288	0.82	0.41
small & medium Dialogic, Interactive - large Dialogic, Interactive	-4.72	20.16	288	-0.23	0.81
small & medium Other - large Other	-15.34	20.16	288	-0.76	0.45