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BACTERIAL, FUNGAL AND VIRUS MOLECULAR BIOLOGY - REVIEW

How microbiological tests reflect bacterial pathogenesis and host adaptation

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Abstract
Historically, clinical microbiological laboratories have often relied on isolation of pure cultures and phenotypic testing to identify microorganisms. These clinical tests are often based on specific biochemical reactions, growth characteristics, colony morphology, and other physiological aspects. The features used for identification in clinical laboratories are highly conserved and specific for a given group of microbes. We speculate that these features might be the result of evolutionary selection and thus may reflect aspects of the life cycle of the organism and pathogenesis. Indeed, several of the metabolic pathways targeted by diagnostic tests in some cases may represent mechanisms for host colonization or pathogenesis. Examples include, but are not restricted to, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella enterica*, *Shigella* spp., and enteroinvasive *Escherichia coli* (EIEC). Here, we provide an overview of how some common tests reflect molecular mechanisms of bacterial pathogenesis.

Keywords Bacteria · Metabolism · Pathogenesis · Diagnostic tests · Bacterial culture

Introduction

One critical hurdle for the launch of microbiology as a scientific discipline was the development of methods for the growth of microorganisms in pure cultures. In 1860, Louis Pasteur pioneered the use of a liquid culture medium to grow bacterial cultures in the laboratory. Later, the importance of growing bacteria on solid media was recognized as this allows for physical separation of individual colonies and the isolation of pure cultures. Early attempts of solid culture techniques relied on natural materials such as potato slices. Later, gelatin was added to media as a solidifying agent. In the 1880s, Angelina Fannie Hesse and Walther Hesse, both working in Robert Koch's laboratory, used agar–agar as gelling agent for bacterial growth on top of and inside the solidified media. Compared with gelatin, agar offered greater resistance to microbial degradation and it remained solid at temperatures required for bacteria to grow [1, 2]. Julius Richard Petri further improved these methods in 1887 when he proposed the use of glass dishes, now known as Petri dishes, to reduce contamination [3].

Bacterial growth under laboratory conditions depends on different factors, such as nutrient availability, pH, temperature, and presence of oxygen or other gases [4, 5]. The composition of the media determines whether it is a general purpose growth media or if it is an enriched, selective, or differential media. While a general media supports growth of a large variety of bacteria, enriched media are formulated to support growth of even the most fastidious organisms. Selective media, on the other hand, are media supplemented with dye or antibiotic that inhibit growth of some microorganisms and thus allow the recovery of specific genera. This media is different than a differential media, which contains some ingredients that allow identification of specific groups of microorganisms from a mixed culture [6]. Often, the isolation of microorganisms from clinical samples relies on a combination of enrichment (liquid) media and selective or differential (solid) media.

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To have diagnostic value, any method to isolate or differentiate organisms must be based on properties that are highly conserved in the bacterium of interest. Furthermore, these properties should be absent from other microorganisms that are commonly found in the specimen. While the presence of such phenotypic traits has practical value for testing, it is conceivable that these conserved traits might be under evolutionary selection and therefore possibly involved in pathogenesis and host transmission. In this review, we use selected examples to illustrate how microbiological tests reflect important aspects of bacterial pathogenesis.

Staphyloxanthin produced by *Staphylococcus aureus*

Pigments can provide simple clues to identify or distinguish certain microbes and have on occasion been applied to taxonomy. *Staphylococcus aureus* produces a golden-yellow carotenoid pigment, staphyloxanthin. The species name *aureus*, Latin for "golden," refers to the color of colonies. In 1884, Rosenbach identified *S. aureus*, which he discriminated against from the closely related microbe *Staphylococcus alba*, with *alba* being Latin for "white," based on pigmentation [7]. It was later realized that this classification method was somewhat inaccurate as pigmentation is multifactorial, dependent on growth conditions, and variable even between members of the same species [8]. Therefore, more reliable methods based on other characteristics of *S. aureus*, such as coagulase production, replaced this method of identification even though more than 90% of *S. aureus* human isolates have a golden pigmentation [9].

S. aureus is a frequent member of the human microbiota and causes a broad range of diseases such as soft tissue infections, respiratory infections, and food poisoning. The organism is commonly found in the skin, nostrils, or vagina and an estimated 20–30% of the human population are colonized by *S. aureus* [10, 11]. It is an important cause of hospital-acquired infections, with around 500,000 infections and around 50,000 deaths in the USA each year [10]. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-intermediate/resistant MRSA (VISA/VRSA) is of great clinical concern. Pathogenic strains often cause disease through the production of an arsenal of virulence factors consisting of hemolysin, coagulase, enterotoxins, protein A, and pigmented carotenoids such as staphyloxanthin.

S. aureus produces multiple pigmented carotenoids through a well-studied biosynthetic pathway that culminates with the production of staphyloxanthin [8, 12]. Pigmented strains have a wider environmental distribution and survive longer on inanimate surfaces than their non-pigmented counterparts [13]. Loss of pigmentation decreases *S. aureus* virulence in a murine skin abscess model and a systemic model of infection [14]. During infection, staphyloxanthin promotes bacterial survival by conferring relative resistance to inflammatory reactive oxygen species (ROS). Like other carotenoid pigments, staphyloxanthin has numerous conjugated double bonds that can be readily oxidized and thus detoxify ROS [15]. Defined *S. aureus* mutants unable to produce staphyloxanthin are more susceptible (about one to two orders of magnitude) to killing by hydrogen peroxide, superoxide radical, hydroxyl radical, hypochlorite, and singlet oxygen under laboratory conditions and by mouse and human neutrophils [14, 16–18]. The fitness defect of staphyloxanthin-deficient mutants was rescued in the context of gp91phox (CYBB) deficiency. gp91phox is the catalytic subunit of the phagocyte oxidase complex (PHOX) and required for inflammatory ROS production. Interestingly, when staphyloxanthin biosynthesis genes were introduced into *Streptococcus pyogenes* (Group A *Streptococcus*), the now pigmented bacteria had increased antioxidative activity and resistance to neutrophils. Furthermore, the transformants were also capable of producing larger lesions in a murine skin infection model [14, 19]. Taken together, these experiments provide strong evidence that staphyloxanthin confers resistance to the oxidative burst of inflammatory cells.

Staphyloxanthin has been evaluated as a potential drug target to treat *S. aureus* infection [20]. Pharmacological inhibitors of the carotenoid biosynthesis pathway have been shown to increase the susceptibility of *S. aureus* to singlet oxygen and killing by murine whole blood [14]. Natural compounds that inhibit staphyloxanthin biosynthesis, such as rhodomyrtone, also increase the susceptibility of *S. aureus* to ROS [21, 22]. The dehydrosqualene synthase of *S. aureus* shares high homology to the human squalene synthase involved in cholesterol biosynthesis. Drugs designed to target human squalene synthase, primarily developed to manage high blood cholesterol levels, also blocked staphyloxanthin production in *S. aureus*, and reduced colonization in a mouse infection model [16], demonstrating the potential for targeting staphyloxanthin biosynthesis as an anti-virulence approach to treat *S. aureus* infections.

Pyocyanin production by *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is one of the most common Gram-negative strains isolated in the clinical microbiology laboratory. *P. aeruginosa* produces pyocyanin, a blue, redox-active phenazine derivative. Although *P. aeruginosa* and other *Pseudomonas* spp. all have the capability to produce pyoverdine [23], a siderophore with fluorescent properties, *P. aeruginosa* is the only known organism capable of

producing pyocyanin. Pyocyanin production by *P. aeruginosa* on TECH agar, also referred to as *Pseudomonas* P agar, is a distinguishing feature to discriminate between *P. aeruginosa* and other *Pseudomonas* spp. In 1900, King et al. developed the TECH agar for improved production of pyocyanin [24]. In the past, pyocyanin production has been used as a sole indicator of the presence of *P. aeruginosa*; however, there are some limitations. For instance, pyoverdine may appear yellow-green, which could be misinterpreted as the green–blue color of pyocyanin [23]. Moreover, *P. aeruginosa* strains occasionally do not produce pigment. Since the early 1990s, laboratories have used a complex biochemical test to identify *P. aeruginosa* [25–27].

Chronic colonization with *P. aeruginosa* is associated with decreased pulmonary function and poorer prognosis. Interestingly, *P. aeruginosa* strains isolated from cystic fibrosis patients produce more pyocyanin than other clinical isolates [28], suggesting a potential role for pyocyanin production in host colonization in cystic fibrosis patients. During infection, this exotoxin is produced in concentrations up to 100 µmol/L [29]. In a mouse model of lung infection, biosynthesis of pyocyanin is required to establish lung colonization and pathology [30]. The excessive production of inflammatory ROS triggered by pyocyanin is a main cause of tissue damage [30–32]. As a redox-active compound, pyocyanin manipulates, directly or indirectly, a number of host processes such as cellular respiration, ciliary beating of airway epithelial cells [29], nitric oxide production by macrophages and endothelial cells [33], apoptosis in neutrophils [34, 35], and calcium homeostasis in airway epithelial cells [36]. Bacterial colonization in cystic fibrosis patients often involves a polymicrobial community. In addition to interfering with host cell functions, pyocyanin released *P. aeruginosa* inhibits growth of competing microbes through a non-enzymatic redox cascade via NADH or NADPH [37].

Capsule production by *Klebsiella pneumoniae*

Klebsiella spp. are Gram-negative bacteria belonging to the *Enterobacteriaceae* family. *Klebsiella* spp. are commonly differentiated base on their biochemical reactions. They produce lysine decarboxylase, but not ornithine decarboxylase, and are typically positive in the Voges-Proskauer test. In addition to biochemical tests, colony morphology can provide insights into the identity of a microorganism. *K. pneumoniae* isolates usually express a complex capsule, resulting in a characteristic mucoid phenotype readily recognizable on lysogeny broth agar plates [38, 39].

Klebsiella spp. inhabit the environment and mammalian mucosal surfaces, such as the intestinal tract [40–42]. *Klebsiella* spp. are opportunistic pathogens and a significant cause of healthcare-associated infections, mainly in immune-compromised individuals [43]. The majority of infections are mainly caused by *Klebsiella pneumoniae*, followed by *Klebsiella oxytoca*, with *K. pneumoniae* causing 8% of all nosocomial bacterial infections in the USA [43]. The exopolysaccharide capsule is one of the main virulence factors in *K. pneumonia* (recently reviewed in [44]). The exact chemical composition and structure of the polysaccharide capsule vary, giving rise to more than 77 different capsule serotypes. Bacterial capsule production promotes environmental survival by increasing resistance to desiccation [45]. Within the host, the capsular polysaccharide protects against phagocytosis by alveolar as well as immature macrophages and impedes tissue clearance in a murine pneumonia model [46, 47]. Capsule production also affects dendritic cell differentiation [47]. Furthermore, the extracellular capsule prevents bacterial cell lysis by preventing humoral host defense molecules such as antibodies and the complement to access to the outer membrane [48, 49]. *Klebsiella* mutants unable to produce an exopolysaccharide capsule are readily killed by the complement system [48]. Only a few examples of the K antigens have been intensively studied [50, 51]. Strains expressing K1 and K2 are considered especially virulent. K2 capsule type is the most common serotype isolated from patient with an UTI and represents the predominant serotype in human clinical isolates, but it is rarely found in the environment [52, 53].

In addition to lung infections, *Klebsiella* causes pyogenic liver abscesses [54]. When cultured under laboratory conditions, *Klebsiella* isolates associated with pyogenic liver abscesses exhibit a mucoid, hypermucoviscosity colony phenotype. Hypermucoviscosity is assessed using a string test. When a bacterial colony is touched with a inoculation loop and a mucoviscous string extends for more than 5 mm when moving the loop up indicates a positive test [55]. The hypermucoviscous phenotype was initially correlated to an RmpA-mediated increase in capsule production [56]. Indeed, the regulatory protein RmpA is required for the activation of capsule gene expression [54]. The strong correlation between the presence of *rmpA* and hypervirulence is currently used as a biomarker for the identification of hypervirulent *K. pneumoniae*. However, the exact mechanism of how *rmpA* contributes to hypermucoviscosity and hypervirulence is still under investigation. Moreover, it was recently shown that a small protein, RmpD, is essential for hypermucoviscosity, yet this gene is not involved in capsule production [57]. As such, hypermucoviscosity and hypervirulence may be two distinct phenomena and further research is needed to clarify the relationship between these two phenomena.

Tetrathionate respiration by *Salmonella*

The *Enterobacteriaceae* family contains many genera that are genetically related but differ in their metabolic traits. For example, utilization of the oxidized sulfur compound tetrathionate ($S_4O_6^{2-}$) is a common approach to enrich for entero-pathogenic *Salmonella enterica* serovars from complex biological specimens. Tetrathionate metabolism is phylogenetically widespread in the environment, while tetrathionate-reducing organisms in the intestinal tract are rare [58, 59]. Tetrathionate enrichment, developed by Müller in 1923, is based on the ability to utilize tetrathionate as a terminal electron acceptor [60]. The addition of tetrathionate to the enrichment media stimulates the growth of tetrathionate reducing bacteria, such as *Salmonella* [61, 62]. The tetrathionate reductase, encoded by the *ttrSRttrBCA* gene cluster in *Salmonella*, catalyzes the reduction of tetrathionate to two molecules of thiosulphate [63]. Later, it was then noted that members of the *Proteus* group are tetrathionate reducers as well. Jeffries described that the addition of novobiocin at 40 mg per liter of broth to suppress the growth of *Proteus* spp., which improved the selectivity of the media [64]. Modified tetrathionate media is used in the clinical diagnostic laboratory for the isolation of *Salmonella* from food, environmental samples and animal feces (**Fig. 1**).

The observation that tetrathionate respiration is highly conserved among most *Salmonella* strains has led to the speculation that it might be an important trait related to the lifecycle of the organism [63], such as survival of an environmental stage in soil. Recently, it was shown in animal models that tetrathionate respiration contributes to colonization of the gut lumen during infection [65]. Reactive oxygen species generated by the inflammatory host response oxidize the thiosulfate present in the gut lumen, thus creating tetrathionate. Mutants unable to utilize tetrathionate poorly colonize the intestinal tract [65]. Gut colonization

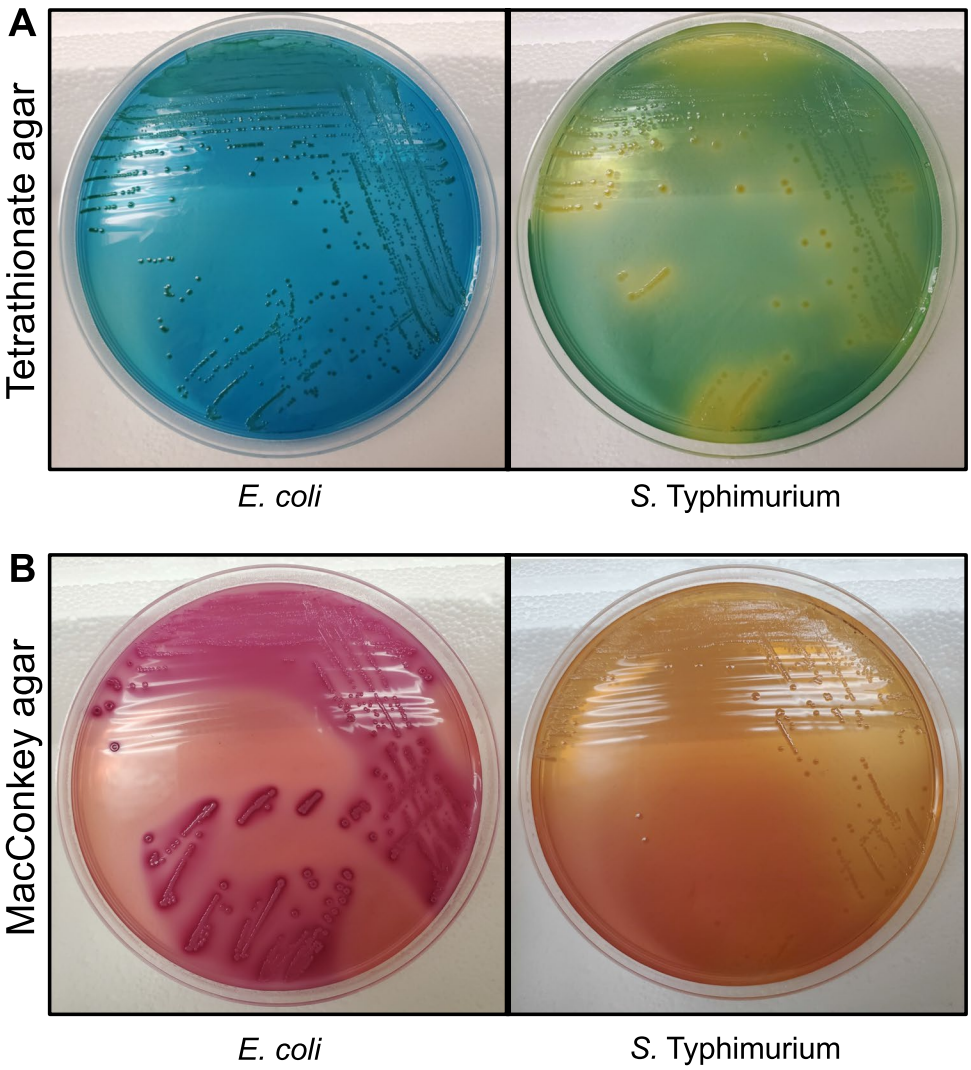

Fig. 1 Bacterial utilization of tetrathionate and lactose **(a)** *E. coli* and *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) were grown on tetrathionate agar plates (peptone 10 g/l, NaCl 5 g/l, $K_2S_4O_6$ 5 g/l, bromothymol blue 0.05 g/l, agar 15 g/l) at 37 °C. When tetrathionate is reduced by *S.* Typhimurium, the media is acidified and the color of the pH indicator bromothymol blue changes from blue to yellow. (**b**) *E. coli* and *S.* Typhimurium were streaked on MacConkey agar plates (Pancreatic digest of gelatin 17 g/l, peptone 3 g/l, lactose 10 g/l, bile salts 1.5 g/l, sodium chloride 5 g/l, neutral red 30 mg/l, crystal violet 1 mg/l, agar 13.5 g/l) and incubated at 37 °C. Lactose fermentation by *E. coli* is indicated by a color change pH indicator neutral red from yellow to pink or red

and fecal shedding are critical for fecal–oral transmission of non-typhoidal *Salmonella* strains in a mouse model [66], and as such, tetrathionate respiration and other respiratory pathways likely contribute to successful host transmission [67].

Pathoadaptation, enteroinvasive *E. coli* (EIEC) and *Shigella*

Shigella and enteroinvasive *Escherichia coli* (EIEC) are Gram-negative bacteria that cause bacillary dysentery. *Shigella* and EIEC likely originated between 35,000 and 270,000 years ago from various ancestral *E. coli* [68]. EIEC and *Shigella* subgroups may have developed independently from commensal *E. coli* through convergent pathoadaptive evolution that included the acquisition of virulence genes, followed by loss of functional genes that interfere with the virulence and/or with the intracellular lifestyle of the bacteria. Although classified as different species, molecular evidence and phylogenetic analysis indicate that *Escherichia coli* and all members of the genus *Shigella* are same species, yet form distinct pathovars [68]. EIEC is thought to be a more recently derived lineage at an intermediate stage in the pathoadaptive process than *Shigella* strains. It is also possible that EIEC is evolving independently from *Shigella*, with differing selective pressures towards a lifestyle that allows for both replication within commensal and epithelial mucosal niches [69]. Due to this convergent evolution and similar lifestyles shared by EIEC and *Shigella*, these two groups of organisms are difficult to distinguish due to nearly identical physio-biochemical properties. *Shigella* and EIEC strains are non-motile and always lysine decarboxylase (LDC) negative. In contrast, non-invasive *E. coli* strains are mostly motile and LDC positive. One notable difference between *Shigella* and EIEC is that EIEC ferments xylose and produces gas from glucose, while *Shigella* does not.

During their evolution from an extracellular commensal of the mammalian gut to an intracellular pathogen, EIEC and *Shigella* acquired a virulence plasmid, which encodes genes required for host cell invasion and intracellular replication [70]. The acquisition of the virulence plasmid by the ancestral species of *E. coli* is followed by convergent pathoadaptive processes between EIEC and *Shigella*, such as the accumulation of pseudogenes and inactivation of anti-virulence genes (AVGs) [71]. Several AVGs identified in *Shigella*, such as those responsible for lysine decarboxylase activity, have also been identified in EIEC [72]. The *cadA* gene encodes for LDC, which is present in virtually all non-enteroinvasive *E. coli* strains. The product of lysine decarboxylation is the polyamine cadaverin. Cadaverin inhibits enterotoxin activity and blocks transepithelial migration of Shigella [73–76]. These processes are essential for *Shigella*/EIEC host colonization. Because of the inhibitory effect of cadaverin on *Shigella* virulence, lysine decarboxylase activity appears to be under negative selection [73–76]. Interestingly, sequence analysis of four different lineages of *Shigella* showed distinct genetic arrangements in each of the strains studied, suggesting that inactivation of *cadA* occurred independently in each of the lineages. These findings highlight the selective pressure towards the loss of LDC activity and increased virulence [75]. While LDC is a trait to discern between enteroinvasive and non-invasive species of *E. coli* [77], the LDC trait alone cannot differentiate between *Shigella* and EIEC.

A better understanding of bacterial pathogenesis and host adaptation can also provide insight into the development of tools that allow for identifying closely related bacteria such as *Shigella* and EIEC. Despite the similarities between *Shigella* and EIEC, the metabolic activity of EIEC resembles that of non-invasive *E. coli* more than it does *Shigella*, which is believed to have undergone significantly more pathoadaptation. Therefore, it is not surprising that many anti-virulence loci characterized and known to be inactivated in *Shigella* remain functional in some strains of EIEC. These emergent pathoadaptive traits, such as the requirement for exogenous nicotinic acid, altered intracellular levels of polyamines like spermidine, and lactose fermentation, likely represent an intermediate step in the pathoadaptive process and could prove useful to differentiate between *Shigella* and EIEC. Lactose fermentation is a hallmark of *E. coli* metabolism and is commonly used for detecting *E. coli* by culture methods such as MacConkey agar (Fig. 1). The lactose permease gene *lacY* is present in the genome of most *E. coli* strains but missing in *Shigella*. EIEC on the other hand, displays a varying capacity to ferment lactose. Lobersli et al. developed a rapid duplex RT-PCR based method for the *lacY* and the invasion plasmid antigen H gene *ipaH* to differentiate between EIEC and *Shigella* [78]. Similarly, the Kligler's Iron Agar (KIA), which tests the abilities to ferment glucose and lactose to acid and acid plus gas, takes advantage of the varying ability of EIEC to ferment lactose. This test is commonly used to differentiate lactose-fermenting *Enterobacteriaceae* such as *E. coli* from strains that are unable to do so, like *Shigella* [78, 79].

Nicotinamide adenine dinucleotide (NAD) is a crucial cofactor in many metabolic reactions. Quinolinic acid is a precursor for NAD, which *E. coli* can salvage from the environment or obtain through a de novo synthesis pathway. *Shigella*, on the other hand, relies on importing nicotinic acid and converting it to the NAD precursor nicotinic acid mononucleotide [80]. This phenotype depends on the inactivation of the *Shigella nadA* and *nadB* genes, which encode for quinolinate synthetase A and L-aspartate oxidase, respectively. The inactivation of the *nadA* and *nadB* genes is possibly driven by convergent pathoadaptive evolution imposed by accumulation of quinolinic acid, which

was shown to attenuate *Shigella* invasiveness, intracellular dissemination, and transepithelial migration [68, 81, 82]. In contrast to *Shigella*, only some EIEC strains require nicotinic acid for growth, supporting the hypothesis that EIEC might represent an earlier point in the pathoadaptive process than *Shigella*. However, the determination of a requirement for nicotinic acid may provide a useful criterion to differentiate *Shigella* from EIEC.

Conclusions and remarks

Classical microbiological techniques to enrich and identify microorganisms rely on unique and conserved metabolic, phenotypic, or genetic traits. As illustrated above, these traits may directly or indirectly relate to the lifestyle and virulence factors of the pathogen of interest. While newer methods such as DNA sequencing, mass spectrometry, antibody-based staining and agglutination techniques are replacing culture-based methods, we still have a largely unrecognized record of historical studies that have surveyed and reported unique traits of microorganisms. Since classical microbiological tests rely on distinctive traits, we speculate that these techniques could be useful starting points for studying mechanisms of bacterial infections. Future experimental work may yet solve mysteries that have puzzled microbiologists for decades.

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Conflict of interest S.E.W. is listed as an inventor on patent US10092596B2, which describes a treatment to prevent the inflammation-associated expansion of Enterobacteriaceae. The other authors have no additional financial interests.

References

1. Hitchens AP, Leikind MC (1939) The Introduction of Agar-agar into Bacteriology. J Bacteriol 37(5):485–493. https://doi.org/10.1128/JB.37.5.485-493.1939
2. Smith JL (1894) A Note on a New Method of Preparing Culture Media. Br Med J 1(1744):1177. https://doi.org/10.1136/bmj.1.1744.1177
3. Petri RJ (1887) Eine kleine Modifikation des Koch'schen Plattenverfahrens. Zentralblatt für Bakteriologie 1:279–280
4. Walker HH, Winslow CE, Mooney MG (1934) Bacterial cell metabolism under anaerobic conditions. J Gen Physiol 17(3):349–357. https://doi.org/10.1085/jgp.17.3.349
5. Taylor AE (1905) On the preparation of salt-free culture media and the growth of bacteria upon them. J Exp Med 7(1):111–118. https://doi.org/10.1084/jem.7.1.111
6. Sullivan MX (1905) Synthetic Culture Media and the Biochemistry of bacterial Pigments. J Med Res 14(1):109–160
7. Rosenbach FJ (1884) Mikro-organismen bei den Wund-Infections-Krankheiten des Menschen. J.F. Bergmann, Wiesbaden
8. Marshall JH, Wilmoth GJ (1981) Pigments of Staphylococcus aureus, a series of triterpenoid carotenoids. J Bacteriol 147(3):900–913. https://doi.org/10.1128/jb.147.3.900-913.1981
9. Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW (2015) Manual of Clinical Microbiology, Eleventh Edition. American Society of Microbiology. https://doi.org/10.1128/9781555817381
10. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr (2015) Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28(3):603–661. https://doi.org/10.1128/cmr.00134-14
11. Kluytmans J, van Belkum A, Verbrugh H (1997) Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 10(3):505–520. https://doi.org/10.1128/cmr.10.3.505-520.1997
12. Marshall JH, Wilmoth GJ (1981) Proposed pathway of triterpenoid carotenoid biosynthesis in Staphylococcus aureus: evidence from a study of mutants. J Bacteriol 147(3):914–919. https://doi.org/10.1128/jb.147.3.914-919.1981
13. Beard-Pegler MA, Stubbs E, Vickery AM (1988) Observations on the resistance to drying of staphylococcal strains. J Med Microbiol 26(4):251–255. https://doi.org/10.1099/00222615-26-4-251
14. Liu GY, Essex A, Buchanan JT, Datta V, Hoffman HM, Bastian JF, Fierer J, Nizet V (2005) Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. J Exp Med 202(2):209–215. https://doi.org/10.1084/jem.20050846
15. El-Agamey A, Lowe GM, McGarvey DJ, Mortensen A, Phillip DM, Truscott TG, Young AJ (2004) Carotenoid radical chemistry

and antioxidant/pro-oxidant properties. Arch Biochem Biophys 430(1):37–48. https://doi.org/10.1016/j.abb.2004.03.007
16. Liu CI, Liu GY, Song Y, Yin F, Hensler ME, Jeng WY, Nizet V, Wang AH, Oldfield E (2008) A cholesterol biosynthesis inhibitor blocks Staphylococcus aureus virulence. Science 319(5868):1391–1394. https://doi.org/10.1126/science.1153018
17. Clauditz A, Resch A, Wieland K-P, Peschel A, Götz F (2006) Staphyloxanthin plays a role in the fitness of Staphylococcus aureus and its ability to cope with oxidative stress. Infect Immun 74(8):4950–4953. https://doi.org/10.1128/IAI.00204-06
18. Khodade VS, Sharath Chandra M, Banerjee A, Lahiri S, Pulipeta M, Rangarajan R, Chakrapani H (2014) Bioreductively Activated Reactive Oxygen Species (ROS) Generators as MRSA Inhibitors. ACS Med Chem Lett 5(7):777–781. https://doi.org/10.1021/ml5001118
19. Wieland B, Feil C, Gloria-Maercker E, Thumm G, Lechner M, Bravo JM, Poralla K, Götz F (1994) Genetic and biochemical analyses of the biosynthesis of the yellow carotenoid 4,4'-diaponeurosporene of Staphylococcus aureus. J Bacteriol 176(24):7719–7726. https://doi.org/10.1128/jb.176.24.7719-7726.1994
20. Xue L, Chen YY, Yan Z, Lu W, Wan D, Zhu H (2019) Staphyloxanthin: a potential target for antivirulence therapy. Infect Drug Resist 12:2151–2160. https://doi.org/10.2147/IDR.S193649
21. Saising J, Hiranrat A, Mahabusarakam W, Ongsakul M, Voravuthikunchai SP (2008) Rhodomyrtone from Rhodomyrtus tomentosa (Aiton) Hassk. as a Natural Antibiotic for Staphylococcal Cutaneous Infections. J Health Sci 54 (5):589–595. https://doi.org/10.1248/jhs.54.589
22. Lee JH, Park JH, Cho MH, Lee J (2012) Flavone reduces the production of virulence factors, staphyloxanthin and α-hemolysin, in Staphylococcus aureus. Curr Microbiol 65(6):726–732. https://doi.org/10.1007/s00284-012-0229-x
23. Reyes EA, Bale MJ, Cannon WH, Matsen JM (1981) Identification of Pseudomonas aeruginosa by pyocyanin production on Tech agar. J Clin Microbiol 13(3):456–458. https://doi.org/10.1128/jcm.13.3.456-458.1981
24. King EO, Ward MK, Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 44(2):301–307
25. Otto LA, Pickett MJ (1976) Rapid method for identification of gram-negative, nonfermentative bacilli. J Clin Microbiol 3(6):566–575
26. Morris MJ, Young VM, Moody MR (1978) Evaluation of a multitest system for identification of saccharolytic pseudomonads. Am J Clin Pathol 69(1):41–47. https://doi.org/10.1093/ajcp/69.1.41
27. Rosenthal SL, Freundlich LF, Washington W (1978) Laboratory evaluation of a multitest system for identification of gram-negative organisms. Am J Clin Pathol 70(6):914–917. https://doi.org/10.1093/ajcp/70.6.914
28. Le Berre R, Nguyen S, Nowak E, Kipnis E, Pierre M, Ader F, Courcol R, Guery BP, Faure K (2008) Quorum-sensing activity and related virulence factor expression in clinically pathogenic isolates of Pseudomonas aeruginosa. Clin Microbiol Infect 14(4):337–343. https://doi.org/10.1111/j.1469-0691.2007.01925.x
29. Wilson R, Sykes DA, Watson D, Rutman A, Taylor GW, Cole PJ (1988) Measurement of Pseudomonas aeruginosa phenazine pigments in sputum and assessment of their contribution to sputum sol toxicity for respiratory epithelium. Infect Immun 56(9):2515–2517. https://doi.org/10.1128/iai.56.9.2515-2517.1988
30. Caldwell CC, Chen Y, Goetzmann HS, Hao Y, Borchers MT, Hassett DJ, Young LR, Mavrodi D, Thomashow L, Lau GW (2009) Pseudomonas aeruginosa exotoxin pyocyanin causes cystic fibrosis airway pathogenesis. Am J Pathol 175(6):2473–2488. https://doi.org/10.2353/ajpath.2009.090166
31. Lau GW, Hassett DJ, Ran H, Kong F (2004) The role of pyocyanin in Pseudomonas aeruginosa infection. Trends Mol Med 10(12):599–606. https://doi.org/10.1016/j.molmed.2004.10.002
32. Lau GW, Hassett DJ, Britigan BE (2005) Modulation of lung epithelial functions by Pseudomonas aeruginosa. Trends Microbiol 13(8):389–397. https://doi.org/10.1016/j.tim.2005.05.011
33. Shellito J, Nelson S, Sorensen RU (1992) Effect of pyocyanine, a pigment of Pseudomonas aeruginosa, on production of reactive nitrogen intermediates by murine alveolar macrophages. Infect Immun 60(9):3913–3915. https://doi.org/10.1128/iai.60.9.3913-3915.1992
34. Allen L, Dockrell DH, Pattery T, Lee DG, Cornelis P, Hellewell PG, Whyte MK (2005) Pyocyanin production by Pseudomonas aeruginosa induces neutrophil apoptosis and impairs neutrophil-mediated host defenses in vivo. J Immunol 174(6):3643–3649. https://doi.org/10.4049/jimmunol.174.6.3643
35. Usher LR, Lawson RA, Geary I, Taylor CJ, Bingle CD, Taylor GW, Whyte MK (2002) Induction of neutrophil apoptosis by the Pseudomonas aeruginosa exotoxin pyocyanin: a potential mechanism of persistent infection. J Immunol 168(4):1861–1868. https://doi.org/10.4049/jimmunol.168.4.1861
36. Denning GM, Railsback MA, Rasmussen GT, Cox CD, Britigan BE (1998) Pseudomonas pyocyanine alters calcium signaling in human airway epithelial cells. Am J Physiol 274(6):L893-900. https://doi.org/10.1152/ajplung.1998.274.6.L893
37. Hassan HM, Fridovich I (1980) Mechanism of the antibiotic action pyocyanine. J Bacteriol 141(1):156–163. https://doi.org/10.1128/jb.141.1.156-163.1980
38. Nassif X, Fournier JM, Arondel J, Sansonetti PJ (1989) Mucoid phenotype of Klebsiella pneumoniae is a plasmid-encoded virulence factor. Infect Immun 57(2):546–552. https://doi.org/10.1128/iai.57.2.546-552.1989
39. Starr MP (1986) Edwards and Ewing's Identification of Enterobacteriaceae. Int J Syst Evol Microbiol 36(4):581–582. https://doi.org/10.1099/00207713-36-4-581
40. Schaberg DR, Culver DH, Gaynes RP (1991) Major trends in the microbial etiology of nosocomial infection. Am J Med 91(3b):72s–75s. https://doi.org/10.1016/0002-9343(91)90346-y
41. Brown C, Seidler RJ (1973) Potential pathogens in the environment: Klebsiella pneumoniae, a taxonomic and ecological enigma. Appl Microbiol 25(6):900–904
42. Gorrie CL, Mirceta M, Wick RR, Edwards DJ, Thomson NR, Strugnell RA, Pratt NF, Garlick JS, Watson KM, Pilcher DV, McGloughlin SA, Spelman DW, Jenney AWJ, Holt KE (2017) Gastrointestinal Carriage Is a Major Reservoir of Klebsiella pneumoniae Infection in Intensive Care Patients. Clin Infect Dis 65(2):208–215. https://doi.org/10.1093/cid/cix270
43. Podschun R, Ullmann U (1998) Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 11(4):589–603
44. Patro LPP, Rathinavelan T (2019) Targeting the Sugary Armor of Klebsiella Species. Front Cell Infect Microbiol 9:367. https://doi.org/10.3389/fcimb.2019.00367
45. Ophir T, Gutnick DL (1994) A role for exopolysaccharides in the protection of microorganisms from desiccation. Appl Environ Microbiol 60(2):740–745. https://doi.org/10.1128/aem.60.2.740-745.1994
46. Cortés G, Borrell N, de Astorza B, Gómez C, Sauleda J, Albertí S (2002) Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of Klebsiella pneumoniae in a murine model of pneumonia. Infect Immun 70(5):2583–2590. https://doi.org/10.1128/iai.70.5.2583-2590.2002
47. Evrard B, Balestrino D, Dosgilbert A, Bouya-Gachancard JL, Charbonnel N, Forestier C, Tridon A (2010) Roles of capsule and lipopolysaccharide O antigen in interactions of human

monocyte-derived dendritic cells and Klebsiella pneumoniae. Infect Immun 78(1):210–219. https://doi.org/10.1128/iai.00864-09
48. Williams P, Lambert PA, Brown MR, Jones RJ (1983) The role of the O and K antigens in determining the resistance of Klebsiella aerogenes to serum killing and phagocytosis. J Gen Microbiol 129(7):2181–2191. https://doi.org/10.1099/00221287-129-7-2181
49. Jensen TS, Opstrup KV, Christiansen G, Rasmussen PV, Thomsen ME, Justesen DL, Schønheyder HC, Lausen M, Birkelund S (2020) Complement mediated Klebsiella pneumoniae capsule changes. Microbes Infect 22(1):19–30. https://doi.org/10.1016/j.micinf.2019.08.003
50. Arakawa Y, Ohta M, Wacharotayankun R, Mori M, Kido N, Ito H, Komatsu T, Sugiyama T, Kato N (1991) Biosynthesis of Klebsiella K2 capsular polysaccharide in Escherichia coli HB101 requires the functions of rmpA and the chromosomal cps gene cluster of the virulent strain Klebsiella pneumoniae Chedid (O1:K2). Infect Immun 59(6):2043–2050. https://doi.org/10.1128/iai.59.6.2043-2050.1991
51. Arakawa Y, Wacharotayankun R, Nagatsuka T, Ito H, Kato N, Ohta M (1995) Genomic organization of the Klebsiella pneumoniae cps region responsible for serotype K2 capsular polysaccharide synthesis in the virulent strain Chedid. J Bacteriol 177(7):1788–1796. https://doi.org/10.1128/jb.177.7.1788-1796.1995
52. Brisse S, Fevre C, Passet V, Issenhuth-Jeanjean S, Tournebize R, Diancourt L, Grimont P (2009) Virulent clones of Klebsiella pneumoniae: identification and evolutionary scenario based on genomic and phenotypic characterization. PLoS ONE 4(3):e4982. https://doi.org/10.1371/journal.pone.0004982
53. Tsay RW, Siu LK, Fung CP, Chang FY (2002) Characteristics of bacteremia between community-acquired and nosocomial Klebsiella pneumoniae infection: risk factor for mortality and the impact of capsular serotypes as a herald for community-acquired infection. Arch Intern Med 162(9):1021–1027. https://doi.org/10.1001/archinte.162.9.1021
54. Broberg CA, Palacios M, Miller VL (2014) Klebsiella: a long way to go towards understanding this enigmatic jet-setter. F1000Prime Rep 6:64. https://doi.org/10.12703/p6-64
55. Hadano Y (2013) String test. BMJ Case Rep 2013. https://doi.org/10.1136/bcr-2012-008328
56. Yu WL, Ko WC, Cheng KC, Lee HC, Ke DS, Lee CC, Fung CP, Chuang YC (2006) Association between rmpA and magA genes and clinical syndromes caused by Klebsiella pneumoniae in Taiwan. Clin Infect Dis 42(10):1351–1358. https://doi.org/10.1086/503420
57. Walker KA, Treat LP, Sepúlveda VE, Miller VL (2020) The Small Protein RmpD Drives Hypermucoviscosity in Klebsiella pneumoniae. mBio 11(5). https://doi.org/10.1128/mBio.01750-20
58. Barrett EL, Clark MA (1987) Tetrathionate reduction and production of hydrogen sulfide from thiosulfate. Microbiol Rev 51(2):192–205. https://doi.org/10.1128/mr.51.2.192-205.1987
59. Barbosa-Jefferson VL, Zhao FJ, McGrath SP, Magan N (1998) Thiosulphate and tetrathionate oxidation in arable soils. Soil Biol Biochem 30(5):553–559. https://doi.org/10.1016/S0038-0717(97)00177-6
60. Muller L (1923) Un nouveau milieu d'enrichissement pour la recherche du Bacille Typhique at Paratyphique. C R Seances Soc Biol Fil 89:434–437
61. Knox R, Gell PG, Pollock MR (1943) The selective action of tetrathionate in bacteriological media: A report to the Medical Research Council. J Hyg (Lond) 43(3):147–158. https://doi.org/10.1017/s0022172400012766 (**141**)
62. Smith PB, Rhoden DL, Tomfohrde KM, Dunn CR, Balows A, Hermann GJ (1971) R-B enteric differential system for identification of Enterobacteriaceae. Appl Microbiol 21(6):1036–1039
63. Hensel M, Hinsley AP, Nikolaus T, Sawers G, Berks BC (1999) The genetic basis of tetrathionate respiration in Salmonella typhimurium. Mol Microbiol 32(2):275–287. https://doi.org/10.1046/j.1365-2958.1999.01345.x
64. Jeffries L (1959) Novobiocin-tetrathionate broth: a medium of improved selectivity for the isolation of Salmonellae from faeces. J Clin Pathol 12(6):568–571. https://doi.org/10.1136/jcp.12.6.568
65. Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, Russell JM, Bevins CL, Adams LG, Tsolis RM, Roth JR, Baumler AJ (2010) Gut inflammation provides a respiratory electron acceptor for Salmonella. Nature 467(7314):426–429. https://doi.org/10.1038/nature09415
66. Lawley TD, Bouley DM, Hoy YE, Gerke C, Relman DA, Monack DM (2008) Host transmission of Salmonella enterica serovar Typhimurium is controlled by virulence factors and indigenous intestinal microbiota. Infect Immun 76(1):403–416. https://doi.org/10.1128/iai.01189-07
67. Rivera-Chavez F, Zhang LF, Faber F, Lopez CA, Byndloss MX, Olsan EE, Xu G, Velazquez EM, Lebrilla CB, Winter SE, Baumler AJ (2016) Depletion of Butyrate-Producing Clostridia from the Gut Microbiota Drives an Aerobic Luminal Expansion of Salmonella. Cell Host Microbe 19(4):443–454. https://doi.org/10.1016/j.chom.2016.03.004
68. Pupo GM, Lan R, Reeves PR (2000) Multiple independent origins of Shigella clones of Escherichia coli and convergent evolution of many of their characteristics. Proc Natl Acad Sci U S A 97(19):10567–10572. https://doi.org/10.1073/pnas.180094797
69. Lan R, Alles MC, Donohoe K, Martinez MB, Reeves PR (2004) Molecular evolutionary relationships of enteroinvasive Escherichia coli and Shigella spp. Infect Immun 72(9):5080–5088. https://doi.org/10.1128/iai.72.9.5080-5088.2004
70. Schroeder GN, Hilbi H (2008) Molecular pathogenesis of Shigella spp.: controlling host cell signaling, invasion, and death by type III secretion. Clin Microbiol Rev 21(1):134–156. https://doi.org/10.1128/CMR.00032-07
71. Yang F, Yang J, Zhang X, Chen L, Jiang Y, Yan Y, Tang X, Wang J, Xiong Z, Dong J, Xue Y, Zhu Y, Xu X, Sun L, Chen S, Nie H, Peng J, Xu J, Wang Y, Yuan Z, Wen Y, Yao Z, Shen Y, Qiang B, Hou Y, Yu J, Jin Q (2005) Genome dynamics and diversity of Shigella species, the etiologic agents of bacillary dysentery. Nucleic Acids Res 33(19):6445–6458. https://doi.org/10.1093/nar/gki954
72. Casalino M, Latella MC, Prosseda G, Colonna B (2003) CadC is the preferential target of a convergent evolution driving enteroinvasive Escherichia coli toward a lysine decarboxylase-defective phenotype. Infect Immun 71(10):5472–5479. https://doi.org/10.1128/iai.71.10.5472-5479.2003
73. Fernandez IM, Silva M, Schuch R, Walker WA, Siber AM, Maurelli AT, McCormick BA (2001) Cadaverine prevents the escape of Shigella flexneri from the phagolysosome: a connection between bacterial dissemination and neutrophil transepithelial signaling. J Infect Dis 184(6):743–753. https://doi.org/10.1086/323035
74. McCormick BA, Fernandez MI, Siber AM, Maurelli AT (1999) Inhibition of Shigella flexneri-induced transepithelial migration of polymorphonuclear leucocytes by cadaverine. Cell Microbiol 1(2):143–155. https://doi.org/10.1046/j.1462-5822.1999.00014.x
75. Maurelli AT, Fernández RE, Bloch CA, Rode CK, Fasano A (1998) "Black holes" and bacterial pathogenicity: a large genomic deletion that enhances the virulence of Shigella spp. and enteroinvasive Escherichia coli. Proc Natl Acad Sci U S A 95(7):3943–3948. https://doi.org/10.1073/pnas.95.7.3943
76. Day WA Jr, Fernández RE, Maurelli AT (2001) Pathoadaptive mutations that enhance virulence: genetic organization of the cadA regions of Shigella spp. Infect Immun 69(12):7471–7480. https://doi.org/10.1128/IAI.69.12.7471-7480.2001

77. Kott Y (1962) Lysine decarboxylase activity as a simple test in differentiation of Enterobacteriaceae. Nature 196:90–91. https://doi.org/10.1038/196090b0
78. Løbersli I, Wester AL, Kristiansen Å, Brandal LT (2016) Molecular Differentiation of Shigella Spp. from Enteroinvasive E. Coli. Eur J Microbiol Immunol 6(3):197–205. https://doi.org/10.1556/1886.2016.00004
79. Johnson JG, Kunz LJ, Barron W, Ewing WH (1966) Biochemical Differentiation of the Enterobacteriaceae with the Aid of Lysine-Iron-Agar. Appl Microbiol 14(2):212–217
80. Gemski P, Formal SB, Baron LS (1971) Identification of Two Widely Separated Loci Conferring Nicotinic Acid Dependence on Wild-Type Shigella flexneri 2a. Infect Immun 3(3):500–503. https://doi.org/10.1128/IAI.3.3.500-503.1971
81. Prunier AL, Schuch R, Fernández RE, Maurelli AT (2007) Genetic structure of the nadA and nadB antivirulence loci in Shigella spp. J Bacteriol 189(17):6482–6486. https://doi.org/10.1128/jb.00525-07
82. Prunier A-L, Schuch R, Fernández RE, Mumy KL, Kohler H, McCormick BA, Maurelli AT (2007) nadA and nadB of Shigella flexneri 5a are antivirulence loci responsible for the synthesis of quinolinate, a small molecule inhibitor of Shigella pathogenicity. Microbiology 153(7):2363–2372. https://doi.org/10.1099/mic.0.2007/006916-0