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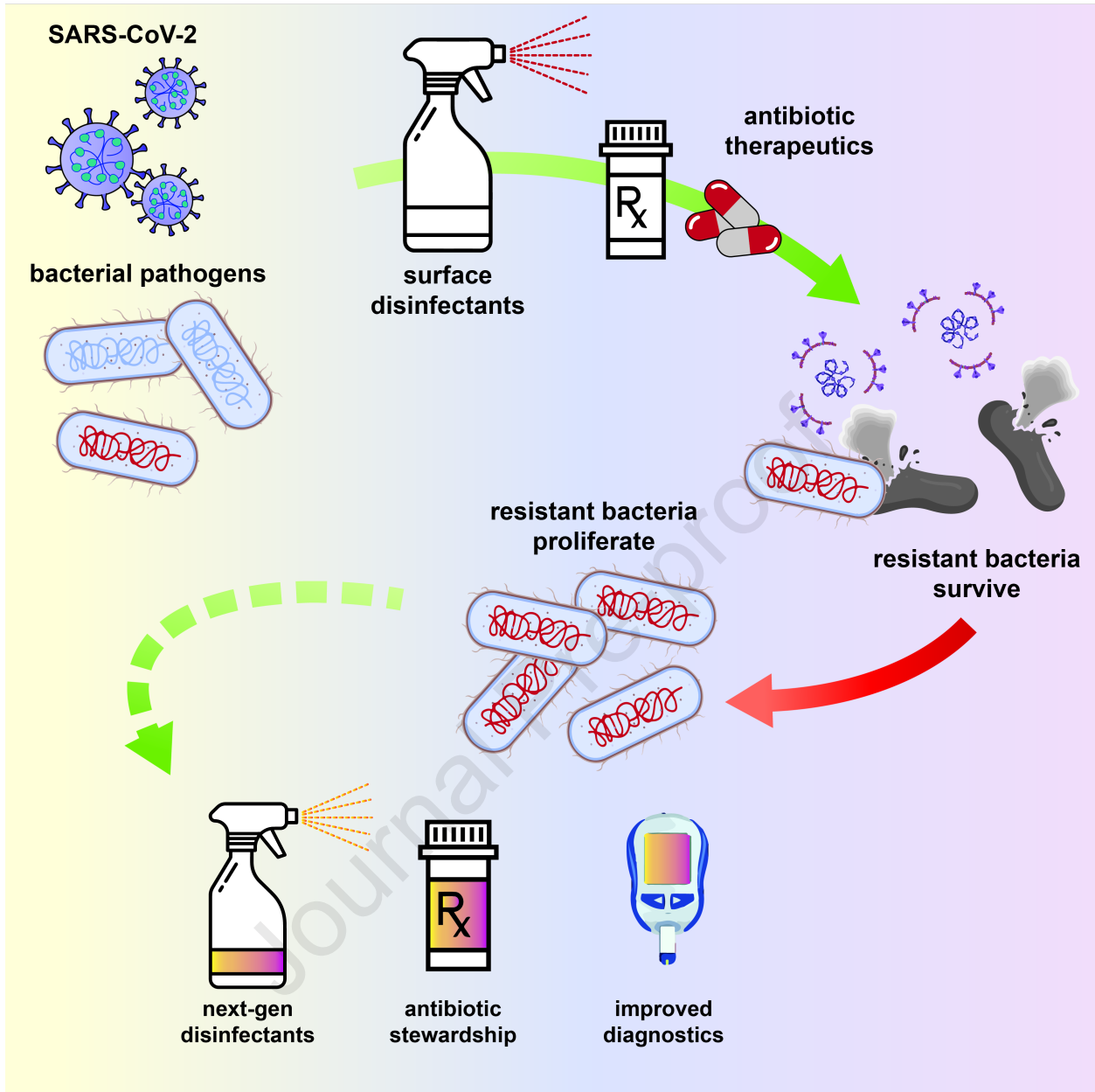
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The Silent Pandemic: Emergent Antibiotic Resistances Following the Global Response to SARS-CoV-2

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Abstract

The ongoing SARS-CoV-2 pandemic has highlighted the importance of the rapid development of vaccines and antivirals. However, the potential for the emergence of antibiotic resistances due to the increased use of antibacterial cleaning products and therapeutics presents an additional, underreported threat. Most antibacterial cleaners contain simple quaternary ammonium compounds (QACs), however these compounds are steadily becoming less effective as antibacterial agents. QACs are extensively used in SARS-CoV-2 related sanitization in clinical and household settings. Similarly, due to the danger of secondary infections, antibiotic therapeutics are increasingly used as a component of COVID-19 treatment regimens, even in the absence of a bacterial infection diagnosis. The increased use of antibacterial agents as cleaners and therapeutics is anticipated to lead to novel resistances in the coming years.

1. Introduction

The global coronavirus disease (COVID-19) pandemic, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has impacted the lives of millions of people worldwide since its initial identification in 2019. As of December 1, 2020, the pathogen has infected more than 97 million people and has caused over 2 million deaths globally (Johns Hopkins University, 2020). In addition to the acute effects of this deadly virus, troubling long-term symptoms that present for months following the initial infection impact an estimated 50%-80% of patients (Greenhalgh et al., 2020). In addition, dangerous bacterial secondary infections are prevalent, especially among those in intensive care due to COVID-19 (Mirzaei et al., 2020). Along with the direct threat of infection to patients, increased use of disinfectants, including hand sanitizers and surface cleaners, is anticipated to cause increased rates of antimicrobial resistance in pathogenic microbes in the coming years (Bengoechea and Bamford, 2020).

The SARS-CoV-2 pandemic has exacerbated the existing global crisis of antimicrobial resistance (AMR) (Mirzaei et al., 2020). An estimated 700,000 mortalities occur annually due to unsuccessful antibiotic treatments, and that number is growing rapidly. In the United States alone, 35,000 deaths are attributed to AMR annually at a cost of \$20 billion in healthcare-related expenses (Services, 2019; O'Neill, 2014). Overuse of both therapeutic antibiotic agents and disinfectants continues to cause emergent resistances in pathogens (Services, 2019; Bragg *et al.*, 2014); at the current rate of resistance-related deaths, it is estimated that mortalities will reach 10 million annually by 2050 (Figure 1a) (O'Neill, 2014). This trend is anticipated to be accelerated by the increased use of antibiotics and antimicrobials due to the current pandemic. The impact of the pandemic on the US, shown as excess mortalities as a result of COVID-19 as compared to other major respiratory illnesses such as influenza and pneumonia, is shown in Figure 1b (CDC, 2020). Here, we summarize two key areas in which COVID-19 is anticipated to yield emergent bacterial resistances: quaternary ammonium compounds used as disinfectants and antibiotic therapeutics often administered unnecessarily with COVID-19 hospitalizations.

2. Quaternary ammonium compound resistance

The antimicrobial potential of quaternary ammonium compounds (QACs) was first identified over a century ago, when researchers at the Rockefeller Institute described the bactericidal activity of a series of hexamethylenetetraminium salts on *Salmonella typhi* in 1916 (Jacobs, Heidelberger, Amoss, 1916). Since then, QACs have come to constitute one of the largest classes of active ingredients in disinfectant and biocide formulations. Due to their potential for activity against a wide variety of pathogens (bacteria, fungi, enveloped viruses, and amoebae), QACs are recommended for general hospital use in the sterilization of noncritical patient care equipment – any surface that has the potential for contact with skin but not mucous membranes (Leas et al., 2015). Outside of hospitals, QACs also see use as surface disinfectants in household and foodservice settings, comprising the active ingredient of many commercially-available cleaning sprays and wipes.

While the general class of QACs may contain a wide variety of substituents around the ammonium cation, the most commonly-employed QACs for sanitization are alkonium chlorides, with benzalkonium (BACs) and dimethyldecyl ammonium chloride (DDAC) being the most common (Figure 2b). The former contains at least one benzyl group in addition to linear or branched alkyl groups ranging from 1 to 18 carbons in length. The most common counterion in QACs by far is chloride, but bromide, saccharide, and acetate are also seen (Hora *et al.*, 2020; Schrank, *Minbiole and Wuest*, 2020). Commercial QAC formulations are often dilute solutions (generally between 0.01% and 1%) and may contain a combination of multiple QACs. Recently, investigations into the structure-activity relationships of simple QAC

scaffolds have revealed that incorporation of multiple ammonium cations and decreasing structural rigidity of QACs may lead to improved efficacy and therapeutic indices (Leitgeb et al., 2020).

While bacterial resistance to QACs was first identified in the 1980s, the precise mechanisms by which this resistance occurs remain significantly understudied (Russell, Hammond and Morgan, 1986; Jennings *et al.*, 2017; Jennings, Minbiole and Wuest, 2016; Jennings *et al.*, 2016). As the vast majority of QAC disinfectants function via membrane permeabilization leading to cell lysis, some bacterial species have intrinsic resistance to QACs caused by the composition of their outer membrane. However, it is generally accepted that acquired resistance in Gram-positive bacteria such as *Staphylococcus* species is the result of the presence of multidrug or QAC-specific efflux pumps (Figure 2a) (Bragg et al., 2014; Sidhu et al., 2002). Studies have shown that methicillin-resistant *S. aureus* (MRSA) isolates containing *qac* genes have significantly higher minimum bactericidal concentrations (MBCs) than those without these genes, confirming that *qac* genes are indeed likely the true mechanism of resistance in these bacteria (Smith et al., 2008). Although QAC resistance in Gram-negative bacteria is less common in the literature, two- to eight-fold changes in minimum inhibitory concentrations (MICs) in Gram-negative species were observed from species isolated from drains that had repeated QAC exposure (McBain et al., 2004). Additional studies have highlighted the emergence of QAC tolerance in the pathogens *Listeria monocytogenes* (Xu et al., 2014) and *Pseudomonas aeruginosa* (Méchin et al., 1999), with the latter noting membrane composition changes that may contribute to this tolerance.

In *Staphylococcus*, the genes encoding for these efflux pumps are located on mobile genetic elements such as plasmids (Bragg et al., 2014; Sidhu et al., 2002). This allows for efficient horizontal gene transfer, resulting in the potential for the rapid spread of QAC resistance between species (Figure 2a). One study found that of 238 human clinical isolates of *Staphylococci* from Norway, 50% were phenotypically-resistant to BAC. Furthermore, the authors suggested this resistance to be a direct consequence of selective pressure resulting from the use of disinfectants (Sidhu et al., 2002). Additional studies have shown that *Staphylococci* isolated from surfaces frequently treated with QACs have resistances to BAC (one the most common QACs), while *Staphylococci* isolated from other, nontreated surfaces remained susceptible to this compound (He et al., 2014), providing additional evidence that it is in fact the use of QACs that drive resistance acquisition.

The ongoing SARS-CoV-2 (COVID-19) pandemic has been implicated in the exacerbation of QAC resistance. As a component of the response to this pandemic, the manufacture, sale, and use of QAC disinfectants have all increased dramatically (Hora et al., 2020). Of the 508 products on the Environmental Protection Agency's list of recommended disinfectants against SARS-CoV-2, 238 contain a QAC as the active ingredient (EPA, 2019). Disinfection is also occurring in more environments during the pandemic; once mostly relegated to indoor high-risk settings, QACs are now being used to disinfect public transportation, public benches, and other outdoor spaces. Critically, QACs are non-volatile and are not generally rinsed off of surfaces after application, enabling these compounds to remain on these surfaces or in the environment at potentially sub-inhibitory levels for extended periods of time.

The bactericidal activity of QACs is highly dependent on the context of use. Factors such as surface composition, temperature, method of application, and dwell time have all been shown to significantly impact the efficacy of sanitizers containing these compounds (Leas et al., 2015). It is likely that, due to the overwhelming number of hospital admissions during the COVID-19 pandemic, increased pressure to ensure rapid room turnaround between patients may have led to a relaxation in sanitization protocols; the pandemic has been linked to shortages in PPE, supplies and testing, and logistical support (McMullen et al., 2020). These challenges have further been linked to increases in hospital-acquired infections (McMullen et al., 2020; Richterman et al., 2020) and increased rates of sepsis (Li et al., 2020; Liu et al., 2020). Furthermore, in non-clinical environments, there is much less oversight of proper disinfection guidelines than in hospitals. Thus, QAC resistance has also been detected in non-clinical settings; testing

S. aureus strains from the surfaces of ATMs in Hong Kong yielded strains containing the QAC resistance genes *qacA* and *qacB* (Zhang et al., 2012).

Reassessment and continuous auditing of disinfection protocols in all workplace and home settings is crucial to minimize the risk of infection and prevent the development of resistances in clinical bacteria. QACs have also been shown to have synergistic antimicrobial effects with phenolic compounds, so multi-component sanitizers may alleviate the immediate threat (Lambert et al., 2003). Finally, future work to investigate the regulation and function of multidrug efflux pumps in more detail is warranted. Previous studies have shown the potential for rational design of QACs to circumvent efflux as a resistance mechanism, and the use of efflux pump inhibitors in combination with QACs like BAC may prolong the useful lifespan of these now-ubiquitous products (Garrison, Mahoney and Wuest, 2020; Morrison *et al.*, 2019).

3. Antibiotic therapeutic resistance

The emergence and spread of bacterial infections that are resistant to antibiotic therapeutics is an increasing global problem. Such antibiotic resistance leads to longer hospitalizations, higher mortality rates, and a significant economic burden (Tyers and Wright, 2019; Unemo and Jensen, 2017; Klein and Hultgren, 2020). Challenges with resistant strains have also limited our ability to treat new and emerging infections (Bush et al., 2011). Although microorganisms naturally acquire resistance mechanisms, the overuse and misuse of available antibiotics has impacted the rate of resistance acquisition (Figure 3a) (Tyers and Wright, 2019). In addition to inappropriate prescription of these therapeutics, improper antibiotic disposal and patient non-compliance have exacerbated the AMR problem. Along with these challenges, novel classes of antibiotics are rare, and resistances have emerged nearly immediately to those that have been introduced. Common classes of antibiotics are shown in Figure 1b. Therefore, to efficiently treat bacterial infections and prevent the continuing spread of the drug resistance, alternative treatment strategies and improved diagnostics must be developed (Tyers and Wright, 2019; Bush *et al.*, 2011). Amongst various approaches to combat and treat resistant infections, multi-drug therapies and repurposing existing treatments could sufficiently improve efficacy and help avert the emergence of new resistances (Tyers and Wright, 2019).

Despite ongoing efforts to decrease the overuse of antibiotic therapeutics, prescribing antibiotics remains inevitable, especially in severe cases such as bacterial co-infections or secondary infections with another illness to ensure that the pathogen load is eliminated (Mirzaei *et al.*, 2020; Lai, Wang and Hsueh, 2020). Lower- and upper-respiratory tract viral infections substantially predispose the patients to bacterial infections by providing more sites for bacterial adhesion, impairing immune responses, and causing cell and tissue damage that facilitate the spread of bacteria and development of an invasive infection (Mirzaei *et al.*, 2020; Lai, Wang and Hsueh, 2020; Morris, Cleary and Clarke, 2017; Lai *et al.*, 2020; Vincent *et al.*, 2020). As a result, the severity of the illness, as well as morbidity and mortality rates, are often significantly increased (Mirzaei *et al.*, 2020). Viral diseases can be complicated by secondary bacterial infections caused by numerous strains, including *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *S. aureus* and *Haemophilus influenzae* (Morris, Cleary and Clarke, 2017; Rawson *et al.*, 2020). It is important to note that bacterial co-infections are less prevalent than secondary infections. In a study of nearly 1,400 COVID-19 patients, only 2.7% were diagnosed with a bacterial infection that co-occurred with the viral infection (Wang et al., 2021). In another study, 5.1% of the cases (5 out of 99) with COVID-19 were reported to have co-infections including *Acinetobacter baumannii* and *Klebsiella pneumoniae* (Chen et al., 2020). In a larger study by Zheng *et al.*, of 221 patients with COVID-19, 25.8% of the patients (57 out of 221) were diagnosed with co-infections and 29.8% of these co-infected cases were reported to have bacterial co-infections (Blasco et al., 2020). Moreover, a review of nine reports

published on hospitalized COVID-19 patients showed that 8% of the cases (62 out of 806) had bacterial/fungal co-infections (Rawson *et al.*, 2020).

In contrast, the increasing number of the COVID-19 cases diagnosed with secondary bacterial infections following the viral infection has become a substantial concern, as it can lead to poorer patient outcomes and longer hospitalizations (Mirzaei *et al.*, 2020; Rawson *et al.*, 2020; Hendaus and Jomha, 2020; Chen *et al.*, 2020). In a study by Huang *et al.*, 9.8% of cases (4 out of 41) with COVID-19 had secondary bacterial infections (Huang *et al.*, 2020). A second study of COVID-19 secondary infections found that 9.3% had a secondary infection (Ripa *et al.*, 2021). Additionally, increased rates of hospital-acquired infections (McMullen *et al.*, 2020; Richterman *et al.*, 2020) and sepsis (Li *et al.*, 2020; Liu *et al.*, 2020) have occurred during the COVID-19 pandemic, emphasizing the importance of accessible testing and diagnostics for both co-infections and secondary infections; such testing has been limited during the pandemic (McMullen, Smith and Rebmann, 2020; Ripa *et al.*, 2021). One key challenge, as can be seen with the highly variable rate of diagnosed co-infections and secondary infections, is that effective diagnostics for these illnesses remain lacking, especially those that can identify bacteria and resistances rapidly, even when hospital resources are stretched or testing is needed in resource-limited settings (McMullen *et al.*, 2020).

In the majority of cases, antibiotic administration and combination therapies have been reported as the most frequently-practiced treatment strategies for co-infections or secondary infections (Lai, Wang and Hsueh, 2020; Rawson *et al.*, 2020). There is also evidence indicating that 70% of hospitalized COVID-19 patients received at least one antibiotic whether or not they were diagnosed with a bacterial infection (Rawson *et al.*, 2020; Nori *et al.*, 2021). In another study on 138 patients with COVID-19, 64.4%, 24.6% and 18.1% of the patients received moxifloxacin, ceftriaxone and azithromycin, respectively (Wang *et al.*, 2020). Taken together, the danger of bacterial secondary infections along with the preemptive administration of antibiotic therapeutics clearly shows an environment ripe for the emergence of new resistances.

Despite the demonstrated efficacy of antibiotic treatments, the increased use of antibiotics in COVID-19 hospitalizations (Rawson *et al.*, 2020; Nori *et al.*, 2021) during this pandemic is anticipated to result in a strong selective pressure on bacterial pathogens to evolve resistances, leading to an increased rate of drug-resistant bacterial infections (Karbelkar and Furst, 2020). Reported bacterial infections in COVID-19 patients include *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (Rawson *et al.*, 2020). These are also among the most prevalent deadly antibiotic-resistant infections, as reported by the CDC (Figure 3c) (Services, 2019; Zhu *et al.*, 2020). As AMR will likely become an even larger challenge in the post-pandemic era, developing novel therapeutic strategies as well as strictly implementing antimicrobial stewardship programs are crucial to combat this emergence. Although treatments are continuously developed for evolving resistances, therapeutic alternatives such as combination therapies, targeted delivery systems, and alternative small molecules are crucial to combat the fast-paced development of AMR (Brooks and Brooks, 2014).

4. Summary & outlook

As highlighted above, increasing levels of antibacterial resistant organisms are inevitable given the extreme circumstances facing us. This increase has been directly shown in studies of COVID-19 patients; in one study from a New York City hospital, it was found that 98% of patients received antibiotics during their hospitalization for COVID-19, and multiple species (*K. pneumoniae*, *P. aeruginosa*, and *E. cloacae*) had a significant (>10%) decline in susceptibility to at least one antibiotic (Nori *et al.*, 2021). As a result, a holistic, all-encompassing approach will be needed to ward off a future wave of pan-resistant

organisms. Effective communication and breakthroughs in scientific research, namely in new therapeutics and diagnostics, are of paramount importance to mitigate the impact of such a wave.

First and foremost (as evidenced by this perspective), there will need to be greater scientific communication of the upcoming threats in order to increase awareness not only to the general public but to governments as well as non-profit organizations and foundations. The latter is essential to provide the necessary funding levels to conduct the tremendous amount of work facing us in the future. CARB-X (Carb-X, 2016), a global non-profit partnership, is one example of an efficient and well-organized effort focused on supporting innovative and necessary antibiotic development. In addition, media campaigns by both the WHO and CDC have increased awareness to the public about best practices for hand-washing and sanitation. This work will need to be extended more broadly to continue educating the community about the perils of antibacterial drug resistance, as in many cases, the severity and speed of this challenge remain poorly understood by the public (Ancillotti et al., 2018; Röing et al., 2020; Wernli et al., 2017).

On the research front, antibiotic stewardship will be essential. To prolong the effective lives of the available antibiotics, three clinical questions should always be answered prior to antibiotic prescription: first, whether the patient is infected with a microbial pathogen; second, whether they need an antimicrobial treatment; and third, what antimicrobial treatment would have the highest efficacy (Burnham et al., 2017). Developing rapid, low-cost, point-of-care diagnostic technologies is urgently needed to answer these questions in a timely manner. Such rapid diagnostic tests must be able to identify bacteria, determine their antimicrobial susceptibility, and facilitate the prescription of the proper narrow-spectrum antibiotics at the point of care. Such easy-to-use sensors will also facilitate diagnosis of asymptomatic patients infected with the resistant pathogens, particularly in nosocomial settings.

In addition to the development of diagnostics for microbial pathogens, engineering assays to rapidly distinguish between viral and bacterial infections would significantly decrease the rate of unnecessary antibiotic therapeutic administration by preventing their preemptive prescription (Burnham et al., 2017). Conventional diagnostics for bacterial infections are either genotypic methods that detect resistance genes or immunological assays such as ELISA (enzyme-linked immunosorbent assay) that are based on antigen-antibody interactions. The major drawback of the traditional techniques is that they are time-consuming and require centralized laboratory facilities (Burnham *et al.*, 2017; Karbelkar and Furst, 2020). Further, the genotypic methods are not capable of detecting antimicrobial susceptibility and emerging resistance patterns for which a gene has not been defined (Burnham et al., 2017). In contrast, biosensing technologies have demonstrated significant potential for rapid point-of-care testing with low-cost instruments (Karbelkar and Furst, 2020). Electrochemical biosensors, particularly, are favorable for their low-cost assembly, rapid detection, quantitative readout, and extremely low limits of detection (Furst and Francis, 2019). We believe electrochemical biosensors incorporated into low-cost, high-throughput platforms will become the gold standard for point-of-care diagnostics to differentiate between viral and bacterial infections, identify bacterial pathogens present in an infection, and determine the susceptibility of bacterial pathogens to multiple antibiotics to prevent a surge of antibiotic resistances during the COVID-19 pandemic and in the post-pandemic era (Karbelkar and Furst, 2020).

Finally, continued research efforts toward antibacterial agents with novel mechanisms of action are necessary. Significant progress has been made over the past years, aided substantially by the CARB-X initiatives and academic endeavors. However, continued discoveries will be necessary. In a similar fashion, refocused studies toward novel antiseptics are also needed. As alluded to above, disinfectant efficacy is being challenged as never before, and resistance to QACs is rising. New cocktails or formulations will be necessary to maintain activity. Recent work in one of our labs (W.M.W.) has demonstrated that QACs containing two or more ammonium moieties (multiQACs) are not as susceptible to the same resistance mechanisms as BAC and might serve as new additives to broaden the efficacy

scope of this heralded class of disinfectants (Forman *et al.*, 2016; Mitchell *et al.*, 2015; Jennings *et al.*, 2016). Work is ongoing to test this hypothesis against a number of multi-drug resistant organisms.

In summary, the current COVID-19 pandemic, caused by the SARS-CoV-2 virus, has brought society to a standstill and caused hundreds of thousands of deaths. The need for disinfection strategies and treatments for deadly infections cannot be understated (Hardie, 2020; Hsu, 2020; Strathdee *et al.*, 2020). Importantly, though, both the disinfection and therapeutic strategies used to combat the spread and severity of COVID-19 are anticipated to exacerbate another global crisis: antimicrobial resistance. The increased use of antibiotic therapeutics and QAC disinfectants during this pandemic is anticipated to result in the rapid emergence of novel, deadly, AMR pathogens. This secondary crisis will only be mitigated with active efforts to improve communication to the public about the risk of these infections, increase funding to research new and alternative disinfectants and therapies to combat the spread of these microbes, and develop precision point-of-care diagnostics to reduce unnecessary antibiotic prescriptions.

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Limitations of the Study

This work was written with information available at the time of publication. Data on the COVID-19 pandemic is constantly being updated.

Resource Availability

Lead contact: Ariel L. Furst (afurst@mit.edu); Materials availability: there are no materials that were generated. Data and code availability: there is no data or code generated unique to this work.

Author Contributions

Writing, Reviewing & Editing, A.R.M. and M.M.S.; Funding Acquisition, W.M.W. and A.L.F.; Supervision, W.M.W. and A.L.F.

Declaration of Interests

W.M.W. has intellectual property covering novel quaternary ammonium compounds. A.L.F. is a member of the Editorial Advisory Board of iScience.

Figures

Figure 1: Antimicrobial resistance and COVID-19 mortalities. a) Projected global annual deaths due to antibiotic resistance by 2050 as compared to the most prevalent causes of mortalities in 2014.(O'Neill, 2014) b) Annual mortalities due to influenza, pneumonia, and COVID-19 in the United States per 100,000 people. Data for 2021 is through the beginning of March. ("Coronavirus Disease 2019 (COVID-19) | CDC," 2020)

Figure 2: Quaternary Ammonium Compound (QAC) resistance and structure. a) Overview of QAC resistance mechanism in *S. aureus*. Once a QAC enters the cell it engages with the negative transcriptional regulator QacR. Upon binding *qacA* is overexpressed resulting in the production and incorporation of QacA efflux pumps on the cell surface to expel QACs. Homologous mechanisms occur in other organisms. b) Structures of common QAC compounds (benzylalkonium chloride (BAC), cetylpyridinium chloride (CPC), didecyldimethylammonium chloride (DDAC)).

Figure 3: Antibiotic therapeutic resistance and bacterial co-infections. a) Overview of therapeutic resistance. b) Structures of common classes of broad-spectrum antibiotics. c) Mortalities in 2017 due to common bacterial infections. (Services, 2019) These infections are prevalent as co-infections and secondary infections with SARS-CoV-2.(Zhu et al., 2020)

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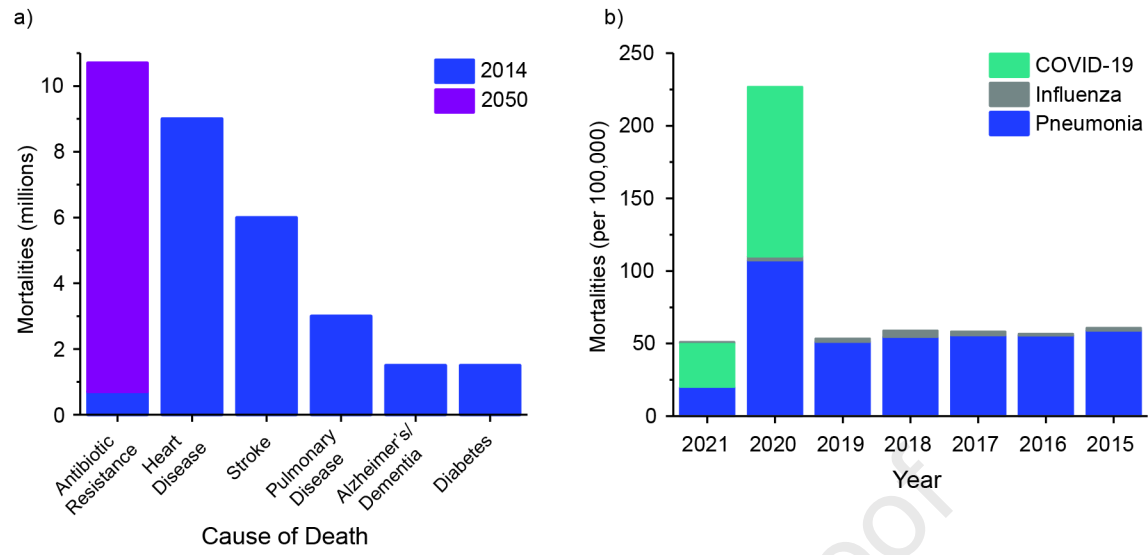
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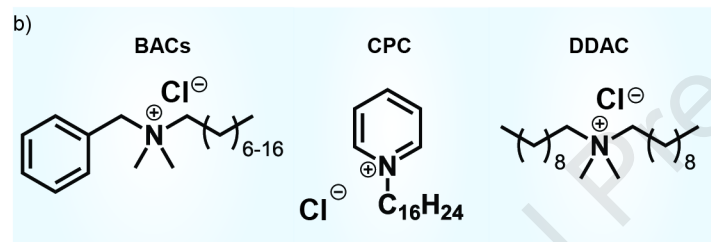
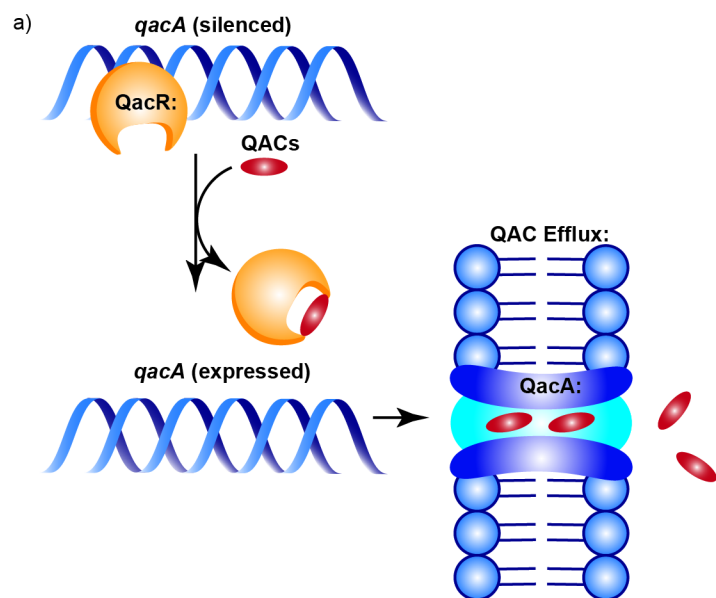
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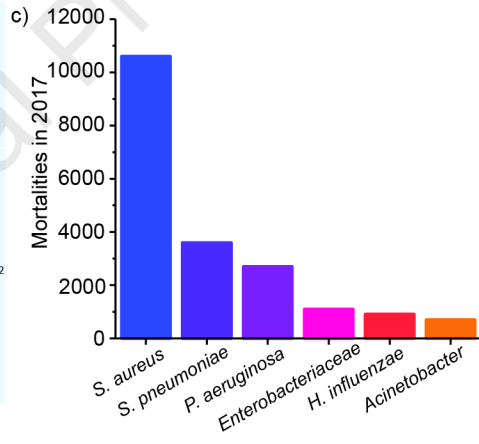
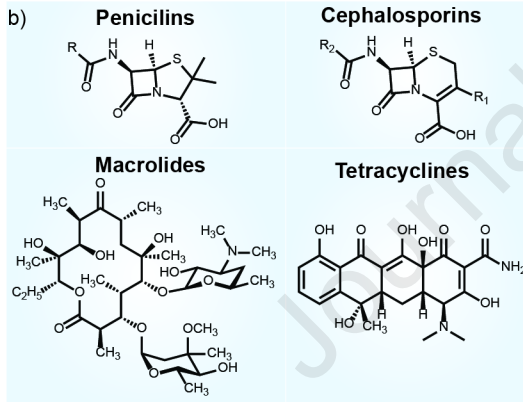
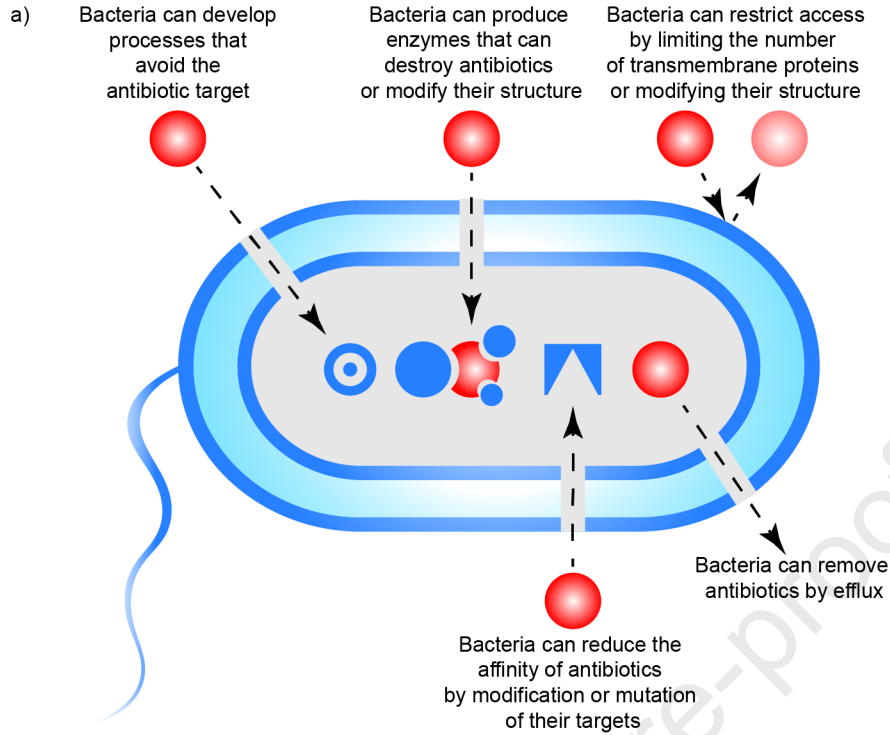
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- Surface disinfectants have been heavily used during the COVID-19 pandemic.
- Antibiotic therapeutics are often prescribed to those hospitalized with COVID-19.
- Their increased use is anticipated to result in emergent antimicrobial resistances.
- Averting AMR requires better disinfectants, antibiotic stewardship, and diagnostics.

Journal Pre-proof