Table of Contents

Introduction

Antibiotic resistance has been increasing among microbial pathogens for decades and has led to the rise of highly antibiotic-resistant "superbugs". With each generation of superbug, the number of acquired antibiotic resistance mechanisms increases and treatment options diminish. We will introduce the three primary classes of Enterococcal superbugs: MRSA, VRE and CRE, antibiotic susceptibility and resistance patterns for each, and new "antibiotic related compounds" being investigated as potential therapeutics against Carbapenem-Resistant *Enterococci* superbugs.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA, also known as "Multidrug-resistant *Staphyloccus aureus*" and "Oxacillin-Resistant *Staphylococcus aureus"* (ORSA), is a strain of *Staphylococcus aureus* that is completely resistant to first line β-lactam antibiotics like amoxicillin, ampicillin, azolcillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, oxacillin, penicillin, piperacillin, temocillin, ticarcillin, and methicillin and potentially resistant to a number of additional antibiotics. In a study of more than 4000 clinical MRSA isolates, collected in India from 2008-2009, a range of antimicrobials were screened and resistance was found at the following rates: erythromycin 70.8%, clindamycin 46.6%, gentamicin 58.3%, co-trimoxazole 55.6%, ciprofloxacin 79.3%, penicillin 100%, no resistance to vancomycin or linezolid was seen (Joshi, S., Ray, P., Manchanda, V., Bajaj, J., Chitnis, D. S., Gautam, V., & ... Balaji, V. (2013)). Though methicillin is no longer prescribed clinically, "methicillin" remains in the name MRSA because resistance to methicillin indicates resistance to the other β-lactam antibiotics.

Methicillin is no longer manufactured for pharmaceutical use. However, TOKU-E manufactures methicillin for *in vitro* research use and companies are now leveraging the near ubiquitous resistance of pathogens to methicillin by making methicillin a main ingredient in diagnostic selective antibiotic cocktails designed to isolate pathogens. Methicillin is an excellent selective media supplement because it effectively eliminates β-lactam-sensitive background growth, facilitating pathogen identification in diagnostic tests.

Methicillin-resistant *Staphylococcus aureus* can be effectively treated with glycopeptide antibiotics like vancomycin and teicoplanin, which block cell wall synthesis through a different mechanism than that used by β-lactam antibiotics, or oxazolidinone antibiotics like linezolid which inhibit bacterial protein synthesis (Joshi, S., Ray, P., Manchanda, V., Bajaj, J., Chitnis, D. S., Gautam, V., & ... Balaji, V. (2013)). MRSA that has acquired glycopeptide resistance is referred to as Vancomycin-resistant *Staphylococcus aureus* (VRSA/VISA). VRSA belongs to the broader family of Vancomycin-Resistant *Enterococci* (VRE) (Alzolibani, Al Robaee, Al Shobaili, Bilal, Ahmad & Saif, 2012).

Vancomycin-Resistant *Enterococci* (VRE)

Vancomycin-resistant *Enterococci*, like other *Enterococci*, colonize the healthy human digestive tract and female genital tract. Infection occurs when these bacteria enter the urinary tract or blood stream, generally through wounds or catheterization. It should be noted that although MRSA can develop vancomycin resistance, not all vancomycin-resistant *Enterococci* are resistant to β-lactam antibiotics. According to the University of California Davis Medical Center treatment algorithm for vancomycin-resistant *Enterococcal* UTIs (urinary tract infections), depending on the type of infection, VRE susceptible to β-lactams can be treated with a number of possible antibiotics: amoxicillin, ampicillin, gentamicin, and streptomycin. Vancomycin-Resistant *Enterococci* that are resistant to β-lactams can be treated with antibiotics like: nitrofurantoin, doxycycline, daptomycin, fosfomycin, linezolid, and quinupristin-dalfopristin (Synercid), depending on the site of infection (Heintz, Cho , Fujioka, Li & Halilovic, 2013). Linezolid is often used as a first line treatment for vancomycin-resistant *Enterococcus faecium* (VREm) (Cattoir & Leclercq, 2012).

Carbapenem-Resistant *Enterobacteriaceae* (CRE)

Carbapenem-resistant *Enterobacteriaceae* are the worst of the "superbugs" because they are resistant to the three major classes of antibiotics traditionally used to fight enterobacterial infections. *E. coli* and *Klebsiella* are two examples of potentially pathogenic *Enterobacteriaceae* which can possess varying degrees of antibiotic resistance. β-lactam antibiotics like penicillins and cephalosporins are the first and second choice antibiotics for treating non-ESBL (extended-spectrum beta-lactamase) enterobacterial infections. ESBL-producing *Enterobacteriaceae* are resistant to both β-lactams and cephalosporins but can be treated with carbapenem antibiotics like doripenem, imipenem, meropenem and ertapenem. Carbapenems are considered the drugs of choice for treating ESBL-producing microbes (Paterson & Bonomo, 2005). Enterobacteria possessing either the NDM-1 or KPC genes are resistant to carbapenem antibiotics.

A 2010 study of 180 NDM-1 containing CRE isolates, gathered from various sites in the UK, India, and Pakistan, revealed very high levels of resistance to all antibiotics except colistin and tigecycline. These CRE isolates were highly resistant to ampicillin, amoxicillin-clavulanate, aztreonam, cefotaxime, ceftazidime, cefepime, cefoxitin, piperacillin, piperacillin-tazobactam, imipenem, meropenem, ertapenem, ciprofloxacin, tobramycin, amikacin, gentamicin, and minocycline (Kumarasamy , Toleman, Walsh, Bagaria, Butt, Balakrishnan… & Woodford, 2010). Two carbapenem hydrolyzing enzymes common to CRE have been identified: KPC (*Klebsiella pneumoniae* carbapenemase) and NDM-1 (New Delhi Metallo-beta-lactamase), or simply MBL (metallo-beta-lactamase) (Farzana, Shamsuzzaman & Mamun, 2013).

A recent study of 35 metallo-beta-lactamase producing superbugs from the Department of Microbiology of Dhaka Medical College in Bangladesh, which included NDM-1 producers, was conducted to assess their antimicrobial resistance patterns. Isolates were screened using an imipenem disk diffusion assay according to CLSI standards. According to their findings,

> "All the MBL producers were 100% resistant to amoxicillin, cephradine, cefuroxime, ceftazidime, cefotaxime, ceftriaxone, gentamicin and piperacillin, 96.77% to amikacin, 93.55% to ciprofloxacin, 87.09% to co-trimoxazole, 80.64% to the combination of taxobactam and piperacillin, and 67.74% to aztreonam (aztreonam is the only β-lactam

> antibiotic that metallo-beta-lactamase producers are unable to hydrolyze). All the MBL-producing isolates were sensitive to colistin (Farzana, Shamsuzzaman & Mamun, 2013)."

Colistin, an older polypeptide antibiotic and member of the polymyxin family, has been used to successfully treat CRE but serious side effects can occur (Koch-Weser, Sidel, Federman, Kanarek, Finer & Eaton, 1970). Research is now being conducted on other polypeptide antibiotics to assess their effectiveness against CRE.

New Antibiotics to Fight Superbugs

The effectiveness of colistin against CRE superbugs has led researchers to look more closely at the polypeptide family of antibiotics as a source of new compounds. Polypeptide antibiotics contain non-protein cyclic polypeptide chains and include bacitracin, polymyxin, colistin and actinomycin. All of these drugs are heterogeneous mixtures of many unique but related antimicrobial compounds. For example, bacitracin, an ingredient in topical antimicrobial ointments like Neosporin®, is comprised of at least eleven related compounds: bacitracin A, A_1, B, B_1, B_2, C, D, E, F, G, X and Xa.

Many of the polymyxin, bacitracin and actinomycin related compounds have yet to be fully characterized for their antimicrobial spectrum, especially with respect to superbugs. Until recently, a significant hurdle was lack of highly purified samples of polypeptide antibiotic related compounds. Mixtures can be tested, but the individual effects of each molecular constituent can be nearly impossible to discern. For example, a mixture of five antimicrobial fractions may show high potency against CRE, but also high toxicity to humans. Testing each molecule individually allows one to characterize each molecule's potency and toxicity profile independently, potentially revealing a high potency, lower toxicity molecule.

TOKU-E has recently released a line of highly purified antimicrobial related compounds designed for use in drug development research called EvoPure®. These include bacitracin A, B_1, B_2, F, Xa, bleomycin A2, B2, cyclosporin A, B, C, D, E, H, L, enramycin A, B, gentamicin A, C1, C1a, C2, C2a, X2, kanamycin A, B, polymyxin B1, B1-I, B2, B3, validamycin A, and vancomycin. A complete list of available antimicrobial related compounds can be found here. TOKU-E is continuously working with researchers to develop new antimicrobial related compounds. For more information, contact us.

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