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REVIEW ARTICLE

Methicillin Resistant *Staphylococcus aureus* and Extended Spectrum Beta-lactamase Producing *Enterobacteriaceae*: A Therapeutic Challenge in the 21st Century

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Abstract: Antimicrobial resistance is one of the greatest global threats to human health in recent times and it limits the achievement of several of the Sustainable Development Goals. Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Extended-Spectrum Beta-Lactamase (ESBL) producing *Enterobacteriaceae* are among the most important multidrug resistant bacterial pathogens. MRSA and ESBL-producing *Enterobacteriaceae* have evolved significantly over the last few decades with important clinical and epidemiological implications. Given the slow progress of development of new antibiotics in recent times, it is likely that these multidrug resistant pathogens will have a greater impact on public health in the 21st Century, unless other effective control measures are instituted. Effective infection control strategies coupled with antibiotic stewardship programs are required to limit the spread and burden of MRSA and ESBL-producing *Enterobacteriacae*.

Keywords: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, Methicillin, ESBL, AMR.

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1. INTRODUCTION

The World Health Organization (WHO) considers Antimicrobial Resistance (AMR) to be one of the greatest threats to human health in the 21st Century. It is estimated that by 2050 if the AMR threat were not properly tackled, it would lead to 10 million people dying every year and a reduction of 2-3.5% in Gross Domestic Product (GDP) with an overall cost of 100 trillion USD [1]. This burden is expected to be relatively higher in sub-Saharan Africa with a drop in GDP of US $2895 billion, representing 20% of the region's total economic output [1]. Antimicrobial resistance limits the achievement of several of the Sustainable Development Goals (SDGs). In particular, SDG3 (Ensure healthy lives and promote well-being for all at all ages) is severely impacted by AMR, as several of the adopted targets in this health-dedicated SDG will be impossible to achieve without the availability of effective antibiotics [2, 3].

Antibiotic resistance, which is the most important aspect of AMR, has been attributed to the misuse and overuse of antibiotics which puts selective pressure on bacterial pathogens leading to the emergence of resistance [3 - 6]. Multidrug Resistance (MDR), the phenomenon where microbes become resistant to several drugs, is now common among many bacterial pathogens. Multidrug resistance is of particular concern as it limits treatment options, can be transferred among bacterial pathogens and enhances morbidity and mortality of the superbugs [3 - 6]. Generally, MDR may occur by one of the two mechanisms. Firstly, the bacteria involved may accumulate multiple resistance genes on plasmids, and each of these genes code for resistance to a single drug [6, 7]. Secondly, multidrug resistance may occur by increased expression of genes that encode multidrug efflux pumps, thereby extruding different types of drugs [6, 7]. In the last few decades, several epidemiologically significant MDR bacterial pathogens have emerged including Methicillin-resistant *Staphylococcus aureus* (MRSA) and Extended-Spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*. MRSA is resistant to all beta-lactam antibiotics and many commonly used antibiotic groups including, aminoglycosides, macrolides, fluoroquinolones, chloramphenicol and tetracyclines [8 - 10]. ESBL-producing *Enterobacteriaceae* are resistant to third generation cepha-

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losporins and monobactams [11]. Together MRSA and ESBL-producing *Enterobacteriaceae* constitute a serious emerging therapeutic challenge in the management of bacterial infections in the 21st century. Though, there is a plethora of review articles on antibiotic resistance, relatively few of them have focused on MDR organisms in recent times; MRSA and ESBL-producing *Enterobacteriaceae* seem to have received some attention, but in many cases, these are covered to limited scope [12 - 14]. To help address some of these gaps, in this paper, we aimed to review the problem of MRSA and ESBL-producing *Enterobacteriaceae* with emphasis on the clinical and epidemiological aspects. This review covers selected relevant articles on antibiotic resistance spanning the period from 1963-2019.

2. METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

Staphylococcus aureus is considered as both a commensal and a human pathogen. As a pathogen, *S. aureus* is implicated in several infections including meningitis, septicaemia, pneumonia, endocarditis and osteomyelitis [15]. Although *S. aureus* can be carried to several body sites as part of the normal flora (commensal), its ecological niche is the anterior nares of the nose [16 - 18]. *S. aureus* occurs principally in the anterior nares because it tends to thrive in conditions of high osmotic pressure and low moisture [19]. It is known that about 50% of the general population are rarely colonized by *S. aureus*, 20% are persistent carriers, while the other 30% carry the organisms intermittently [20 - 22]. *S. aureus* colonization is a major risk factor for the development of invasive disease of the organism in humans [18, 23].

Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant *S. aureus*. However, in the early 1960s, MRSA was observed in several European countries [24 - 28]. MRSA now has a worldwide distribution and is endemic in many hospitals particularly, in Asia, Europe and the United States [29 - 31]. A systematic review of MRSA carriage among healthcare workers in the United States and Europe in 2014 reported a prevalence of 4.6% [32]. In MRSA, the methicillin resistance gene encodes a methicillin-resistant penicillin-binding protein, which is carried on the staphylococcal cassette chromosome *mec*, of which more than ten types have been described so far [33]. In addition to its extensive resistance to antibiotics, MRSA is of serious concern due to the high prevalence of its infections and association with persistent outbreaks, which have serious economic implications [34]. The annual incidence of invasive MRSA infections in the United States is estimated to be 94,360, with 18,650 deaths [35]. Additionally, hospital stays for MRSA infections in the United States cost $14,000, in comparison with $7,600 for all other stays, with twice the length of hospitalization [36]. In Europe, data from thirty-one countries reported 27,711 episodes of MRSA blood stream infections, which were associated with 5,503 deaths and 255,683 days of hospitalization between July 2007 and June 2008. The estimated cost of this length of hospital stay was 44 million Euros [37]. Relatively, there is a scarcity of MRSA data in developing countries, especially on economic costs. In a hospital-based study involving nine African countries, MRSA was detected in 213 (15%) of the 1440 *S. aureus* isolates screened; the prevalence was relatively higher in Cameroon, Kenya and Nigeria (21-30%), and below 10% in Tunisia, Malta, and Algeria [38]. In Asia, the MRSA prevalence is much higher and countries such as Taiwan, Korea and Japan have recorded Healthcare-Associated HA-MRSA prevalence of >40% [39].

Traditionally, MRSA is regarded as a major nosocomial pathogen in healthcare facilities, and is referred to as healthcare-associated MRSA (HA-MRSA) [40, 41]. Only a few of the known HA-MRSA clones are responsible for the majority of infections, and different clones dominate in different geographical regions. For example, the ST239-SCC*mec*III clone predominates in South America, Asia, and Africa [42, 43]. The predominant clone in the United States is CC5-SCC*mec*II (USA100) [44, 45], while in Europe it is CC22-SCC*mec*IV (EMRSA-15) [46 - 50]. It is important to note that the replacement of these clones keep occurring in several geographical regions [51 - 53]. Studies on the evolution of the major HA-MRSA clones indicate strong evidence for a wide range of antibiotic-resistant mutations and mobile genetic elements that are associated with the emergence of these clones in hospital epidemics [54, 55]. Though MRSA is considered a nosocomial pathogen traditionally, it has emerged in the community in the last two decades and is responsible for several types of community-acquired infections [56 - 59]. Epide-

Table 1. Some differences between healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA).

Parameter	HA-MRSA	CA-MRSA
Genetic traits	Various staphylococcal cassette chromosome (most common—USA100, USA200)	Panton Valentine gene, staphylococcal Cassette chromosome IV (most common—USA300, USA400)
Part of body affected	Blood stream; Surgical site; Site of implant	Skin; Lungs
Resistance gene	SCC mec Types I, II, III	SCC mec Type IV, V
Panton–Valentine Leukocidin producer	Rare (5%)	Frequent (almost 100%)
Risk population	Immunocompromised individuals; residency in long term care facilities; recent hospitalizations; dialysis patients; recent surgery	Young, otherwise healthy patients (most common); no recent hospitalizations; anyone
Antibiotic used in management	First-line antibiotics used include vancomycin. Additional newer antimicrobial agents: daptomycin, linezolid and tigecycline.	Doxycycline, clindamycin and cotrimoxazole often used.

Adapted from Popovich *et al*. [29] and Bassetti *et al* [30].

miologically, CA-MRSA and HA-MRSA are considered to be different from each other [60, 61], and Table **1** shows some clinical and genetic differences between them. However, this epidemiological distinction can be blurred by the fact that CA-MRSA and HA-MRSA genotypes are being observed in healthcare and community infections respectively [62]. CA-MRSA infections could also be caused by livestock-associated MRSA (LA-MRSA) [63]. LA-MRSA is initially associated with livestock (such as pigs, cattle, and chicken) and differs genotypically from HA-MRSA and CA-MRSA [63]. Globally, among the known LA-MRSA strains, CC398 is most widely disseminated, followed by CC9 [63].

The advent of Whole Genome Sequencing Analysis (WGSA) has provided us the opportunity of better understanding some of the important MRSA clones. Using WGSA, Strauß *et al*. [64] recently provided insights into the evolution and global dissemination of the ST8 clone (USA 300). The study showed that the ancestor of all ST8 *S. aureus* emerged in Central Europe in the mid-19th century, and then appeared in North America in the early 20th century where it progressively acquired the USA300 features. Starting from North America, USA300 then spread globally, including Africa. In a phylogenetic analysis, Strauß *et al*. [64] demonstrated that the African USA300 isolates formed a monophyletic group within the clade of North American USA300, indicating a single introduction event to the African continent, followed by a spread in the local population [64]. These observations highlight the role international travel may play in the dissemination of antibiotic resistance.

3. EXTENDED SPECTRUM β-LACTAMASE PRODUCING ENTEROBACTERIACEAE

Gram-negative bacteria including *Enterobacteriaceae* have a relatively higher tendency to become antibiotic-resistant partly due to the presence of an outer membrane which excludes antibiotics from penetrating the cell [65]. Addi-tionally, these organisms have a great facility for exchanging genetic material (DNA) that may contain antibiotic-resistant genes among strains of the same species and even among different species [66]. The discovery of antibiotic resistance in Gram-negative bacteria became apparent soon after ampicillin (first semisynthetic penicillin) was clinically introduced in 1961. The first plasmid-mediated β-lactamase in gram-negative bacteria, TEM-1, was detected in *Escherichia coli* isolated from a blood culture from a patient in Greece in 1963 [66]. Over the years, diverse resistance mechanisms have changed the distribution of plasmids and new mobile genetic features have been contributory in the horizontal transmission of resistance genes, with these multiple genes conferring resis-tance to many antimicrobials. Among the *Enterobacteriaceae*, TEM-1 and sulfhydryl variable-1 (SHV-1) β-lactamases were the most prevalent plasmid-mediated enzymes frequently found spreading in countries worldwide [67]. In the 1970s, resistant Gram-negative bacteria had become more common in most hospital-acquired pathogens with TEM-1 and SHV-1 enzymes. Most of these bacteria carried multiple β-lactamases as well as other multidrug-resistant genes. In the early part of the 1980s, a number of new antimicrobials were clinically introduced in the health-care delivery systems, including the third-generation cephalosporins. Due to misuse of these agents, Germany in 1983 experienced the first Extended-Spectrum β-Lactamase (ESBL) in a species of *Klebsiella*. Extended-spectrum β-lactamases are resistance enzymes that usually confer resistance in most Gram-negative bacterial pathogens as a result of more-selective pressure from the use of β-lactams: oxyimino-cephalosporins (such as cefotaxime, ceftriaxone, ceftazidime, or cefepime) and monobactams (aztreonam) but not carbapenems, which had undergone hydrolysis and further mutations [68, 69]. The ESBL enzymes result from a point

Table 2. Beta lactamases classifications, ESBL activity and representative enzymes.

Molecular Class	Functional Group	ESBL Activity[c]	Representative Enzymes
A	2a	N	PC1
	2b	N	TEM-1, SHV-1
	2be	Y	CTX-M-14, -15
	2br	N	TEM-30, SHV-10
	2ber	Y	TEM-50, TEM-121
	2c	N	PSE-4, CARB-3
	2ce	N[e]	RTG-4
	2e	Y	SFO-1, FEC-1, L2
	2f	Y	KPC-2, SME-1[f]
B	3a[g]	Y	IMP, VIM, NDM, L1
	3b	N	CphA
C	1	N	AmpC, ACT-1
	1e	Y	GC1, CMY-37
D	2d	N	OXA-1, OXA-10
	2de	V	OXA-11, OXA-15
	2df	Y	OXA-23, OXA-48

Adapted and modified from Bush [68].

c= Based on hydrolysis of cefotaxime, ceftazidime, or cefepime. e= In spite of k_{cat} values generally B1 s^{-1}, resistance to cefepime and cefpirome is seen in producing organisms. g= Includes subclasses B1 and B3. Y= k_{cat} >5 s^{-1}, N= k_{cat} <5 s^{-1}, V= variable within the functional group.

mutation in the parent β-lactamases, TEM-1 and SHV-1 by one to four amino acid changes which form the basis of resistance presumably due to evolutionary selective pressure from the use of β-lactams, such as oxyimino-cephalosporins and aztreonam. To date, the number of known β-lactamases have increased, and there are now over 1000 that have been identified. The most recognizable among the mutants of SHV-1, named SHV-2, deactivated the extended-spectrum cephalosporin drugs and often carried many other resistance genes on its parent plasmid that conferred reduced susceptibility to other unrelated classes of antimicrobials [70, 71].

Equally important ESBLs of clinical significance are the CTX-M and *AmpC* β-lactamases families, as indicated in Table **2**. The CTX-M family is classified under classes of β-lactamases as class A ESBLs for the past decade, CTX-M–type ESBL enzymes have become most, prevalent in clinical isolates, mostly in *Escherichia coli* isolates in Asia, Europe and South America [72]. Earlier, there were confusions as to where MEN-1 and Tolo-1 enzymes belong. CTX-M-1 was subsequently found to be similar to the MEN-1 enzyme; while CTX-M-44 and CTX-M-45 were known to be the same as Toho-1 and Toho-2 respectively [68, 73]. Since CTX-M-1 recognition in clinical circles in the 1980s, over 130 variants have been identified and genetically classified based on amino acid differences into 5 major divisions, CTX-M-1, -2, -8, -9, or -25 mostly identified in *Escherichia coli* and *Klebsiella pneumoniae* isolates from varying geographical locations [74, 75]. In 1999, a CTX-M-15 variant was recovered from India; belonging to the CTX-M-1 group, it was shown to have dominance in the clinical setting and also shown to have worldwide distribution. Thus, more allelic variants were subsequently recovered from different Gram-negative bacterial isolates in both clinical and community settings and those yet to arrive are a threat to patients' conditions in the clinical environment [72, 76].

AmpC β-lactamases are also of importance and the enzymes can be chromosome or plasmid-mediated. After sequencing of the *Amp*C gene from *Escherichia coli* K-12 strain, it was designated as class C according to Ambler's structural classification of β-lactamases (Table **2**). Thus, differences in molecular structures between β-lactamases classes A and B actually determined *AmpC* gene classification [77]. *AmpC*-like β-lactamases mainly from *Enterobacter* and *Pseudomonas* with ESBLs hydrolyse both penicillins and cephalosporins. Of clinical importance, plasmid-mediated *AmpC* enzymes occurring in Gram-negative bacteria have detection problems with the phenotypic methods, therefore, dissemination associated with ESBLs pose a serious risk of treatment failures [78]. *AmpC* enzymes are inducible, unaffected by EDTA and clavulanic acid inhibitors, usually produced in low quantities and often suppress detection ability. The main mechanisms that initiate acquisition of plasmid-mediated *Amp*C genes and overexpression in bacterial strains are largely due to mutation at the *AmpC* attenuator and promotor regions [79, 80].

A study by [81] earlier reported selective pressure of broad-spectrum cephalosporins such as cefotaxime and ceftazidime as the main cause of production for *AmpC* types of β-lactamases [81]. This suggestion has been entirely modified following a careful re-evaluation on a number of plasmid-mediated bacteria by the same group of investigators recently, that selective pressure of antimicrobials only increase the number of antimicrobial resistance isolates, and that production of *AmpC* β-lactamases largely depend on; suitability of plasmid, stability of bacterial strain interactions, complexity of the plasmids, ability to conjugate freely and survival of plasmid at different conditions [82].

Epidemiological evidence from the SMART study on urinary isolates between 2009 and 2010 showed that ESBL prevalence among *E. coli* and *K. pneumoniae* was 17.6 and 38.9% respectively in Europe, and 8.5 and 8.8% respectively in North America [83]. In both continents, the class A ESBL gene CTX-M-15 was the most prevalent gene (found in >90% of *E. coli* isolates and in 35–65.5% of *K. pneumoniae*), though SHV- and TEM-type genes were also common [83]. Data from the SENTRY Asia Pacific surveillance program reported that CTX-M genes occurred in 38.2–55.5% of *K. pneumoniae* and *E. coli* isolates, and the prevalence of SHV- and TEM-type genes was higher (between 34.3 and 85.3%) [84]. In Africa, a recent review reported ESBL prevalence ranging from 17.7% in Algeria to 82.8% in Cameroon; ESBLs (classes A and D) are common on the continent with the CTX-M-15 gene being most prevalent [85]. The available data on ESBLs show considerable geographical differences in prevalence. For example, a study involving 100 European Intensive Care Units (ICUs) reported that the prevalence of ESBLs in *Klebsiella* ranged from 3% in Sweden to 34% in Portugal [86].

CONCLUSION AND RECOMMENDATIONS

Methicillin-resistant *S. aureus* and ESBL producing *Enterobacteriaceae* have evolved significantly over the last few decades with important clinical and epidemiological implications. Given the slow progress of development of new antibiotics in recent times, it is likely that these MDR bacterial pathogens will have a greater impact on public health in the 21st Century unless other effective control measures are instituted. Effective infection control strategies coupled with antibiotic stewardship programs are required to limit the spread and burden of MRSA and ESBL-producing *Enterobacteriaceae*. Additionally, further studies on the transmission mechanisms and local epidemiology of these two MDR bacterial pathogens are needed. In particular, there is a need for surveillance including molecular epidemiology data on MRSA and ESBL-producing *Enterobacteriaceae* in the developing world, where such efforts have focused mainly on microbes with a greater mortality burden such as *Streptococcus pneumoniae*, *Rotavirus* and *Mycobacterium tuberculosis* [87 - 91].

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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