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Original Article

The lack of sensitivity of methicillin resistant *Staphylococcus aureus* (MRSA) toward teicoplanin

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Abstract

A total of 60 methicillin resistant *Staphylococcus aureus* (MRSA) strains clinical samples in children were collected from cellulitis, abscess, and wound samples in Al-Hilla, and Babylon teaching hospital was identified to species level and antibiotic susceptibility testing with a VITEK-2 system. In the present study, 24 (40%) of MRSA isolates were community- acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA), and 36 (60%) were hospitalized-acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA). The results showed that 35 of female, and 25 of male of MRSA isolates, and these isolates were taken from 7 of infants aged less than 6 months, and 53 children aged between 6 months and 4 years of MRSA isolates. In the present study, antimicrobial susceptibility testing with the VITEK 2 system gave resistant to 10 and sensitive to 6 antibiotics to all bacterial isolates, but only 3(5%) of bacterial isolates were resistant to teicoplanin.

1.Introduction

Methicillin-resistant *S. aureus* (MRSA) infections are now the most common cause of skin abscesses. Community-associated MRSA has also been found to cause severe infections including necrotizing pneumonia, necrotizing fasciitis, purpura fulminans, and severe sepsis in nonimmunocompromised hosts[1]. (MRSA) strains have acquired a gene that makes them resistant to all beta-lactam antibiotics. Hospital-associated strains of this organism are serious nosocomial pathogens that have become resistant to most common antibiotics, and treatment can be challenging. Community-associated MRSA strains occur in people who have not been hospitalized or recently had invasive procedures. They first appeared in high risk populations (e.g., intravenous drug users, people with chronic illnesses), but are now found even in healthy children. Until recently, community-associated strains were susceptible to many antibiotics other than beta-lactams; however, resistance seems to be increasing, and multiple antibiotic resistant strains have started to emerge. Human-adapted MRSA can be transmitted to animals in close contact, which can sometimes act as carriers and re-infect people[2]. MRSA strains have acquired the *mec*A gene, which is carried on a large mobile genetic element called the staphylococcal chromosomal cassette *mec* (SCC*mec*). This gene codes for a penicillin binding protein, PBP2a, which interferes with the effects of beta lactam antibiotics (e.g. penicillins and cephalosporins) on cell walls. It confers virtually complete resistance to all beta-lactam antibiotics including the semi-synthetic penicillins[3]. Acquisition of *mec*A seems to have occurred independently in a number of *S.aureus* strains. Some clonal lineages of *S.aureus* have a tendency to colonize specific species, and may be adapted to either humans or animals. Other lineages ("extended host spectrum genotypes") are less host-specific, and can infect a wide variety of species. Some MRSA strains, called epidemic strains, are more prevalent and tend to spread within or between hospitals and countries. Other "sporadic" strains are isolated less frequently and do not usually spread widely[4]. Hospital-associated MRSA is one of the most prevalent nosocomial pathogens worldwide. Most infections occur in high risk patients, including the elderly and people with open wounds. Infections caused by community-acquired MRSA are also becoming more common. As with many bacterial infections, the case fatality rate differs with the syndrome. Mortality also depends on success in finding an effective antibiotic for the strain[5].

2. Materials and methods

2.1 Bacterial isolates:

Sixty Methicillin-resistant *S. aureus* (MRSA) isolates were obtained from clinical samples in children in Al-Hilla/Iraq during the period from April 2014 to June 2014. Clinical samples were collected from children in Al-Hilla and Babylon teaching hospital in Al-Hilla city, in addition to some private clinic. Clinical isolates were as follows: cellulitis, abscess, and wound samples. These bacterial isolates were identified as *S.aureus* based on their morphology, Gram-staining. Vitek 2 system was performed to identify species level and antibiotic susceptibility testing of *S.aureus* isolates.

2.2 Antimicrobial Susceptibility Test with VITEK 2:

The 0.5 McFarland bacterial suspension was diluted to 1.5 × 107 CFU/ml in 0.45% normal saline. Cards were automatically filled, sealed, and loaded into the VITEK 2 instrument for incubation and reading. The AST-GP67 card used for staphylococci contained benzylpenicillin, oxacillin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, quinupristin/dalfopristin, linezolid, tetracycline, tigecycline, nitrofurantoin, rifampicin, and trimethoprim /sulfamethoxazole.

3. Results and Discussion

Sixty Methicillin-resistant *S. aureus* (MRSA) strains clinical samples were collected from cellulitis, abscess, and wound samples in children in Al-Hilla, and Babylon teaching hospital. 24(40%) of MRSA isolates were community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA), and 36(60%) were hospitalized-acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA). Community associated MRSA strains that express a toxin called PantonValentine leucocidin (PVL) have been linked to skin and soft tissue infections and severe necrotizing pneumonia. It is possible that PVL are associated with increased virulence in general [6]. The two toxins (ETA and ETB) act specifically in the zona granulosa of the epidermis, and even

intraperitoneal inoculation will result in exfoliation. the toxin binds to proteins in keratohyalin granules, and because filaggrins act as intracellular anchors of desmosomes, many investigators have speculated that epidermal splitting is a result of rupture of these desmosomes, probably from proteolytic activity of the toxins [7]. Iyer and Jones, 2003 [8] found that cutaneous abscesses were the most common presentation of cutaneous MRSA infection, definitive treatment consisted of incision and drainage in combination with antimicrobial therapy, the most effective antibiotics were vancomycin, trimethoprim/sulfamethoxazole in combination with rifampin, and linezolid.

The results showed that 35 of female, and 25 of male of MRSA isolates, and these isolates were taken from 7 of infants aged less than 6 months, and 53 children aged between 6 months and 4 years of MRSA isolates. Staphylococcal scalded skin syndrome (SSSS) is blistering skin diseases induced by the exfoliative (epidermolytic) toxins (ET) of *S. aureus.* It is a disease primarily affecting infants and young children, but cases have been reported in adults. It seems that the location of lesions depends on age. In neonates, the lesions are mostly found on the perineum or periumbilically, or both, while the extremities are more commonly affected in older children. The disease begins with erythema and fever, followed by formation of large fluid filled bullae which quickly rupture on slightest pressure to leave extensive areas of denuded skin[9]. Kaplan *et al*, 2005 [10] found that 62% of children with CA-MRSA and 53% of CA-MSSA isolates were admitted to the hospital with skin and soft-tissue, and invasive infections. In this study, antimicrobial susceptibility testing results found that with the VITEK 2 system gave resistant to 10 and sensitive to 6 antibiotics to all bacterial isolates, but only 3(5%) of bacterial isolates were resistant to teicoplanin table (1).

Table (1): Show the results of minimum inhibitory concentration (MIC) and interpretation with the VITEK 2 system

Antimicrobial	MIC	interpretation
Benzylpenicillin	>=0.5	R
Oxacillin	>=8	R
Gentamicin	<=0.5	S
Ciprofloxacin	<=0.5	S
Levofloxacin	0.25	S
Moxifloxacin	<=0.25	S
Erythromycin	>=8	R
Clindamycin	>=8	R
Quinupristin/Dalfopristin	>=16	R
Linezolid	>=8	R
Teicoplannin	>=32	R
Tetracycline	>=16	R
Tigecycline	0.25	S
Nitrofurantoin	256	R
Rifampicin	>=32	R
Trimethoprim/Sulfamethoxazole.	<=10	S

R: represented resistance

S: represented sensitive

The results showed that MRSA isolates were resistant to Benzylpenicillin, and oxacillin. The mechanism of resistance to β-lactam antibiotic is mostly due to either production of β-lactamases that hydrolyze β-lactam ring, or lack of penicillin receptors on cell wall and / or alteration in their permeability to β-lactam antibiotics preventing the uptaking of them [11]. Oxacillin replacs methicillin as oxacillin is stable under storage conditions, and methicillin actually is an excellent inducer of the mecA gene. Staphylococcal resistance to either oxacillin or methicillin occurs when the organism including an altered PBP (PBP2A) that is encoded by the mecA gene [12]. In the present study, the results found MRSA isolates were sensitive to gentamicin. The general mechanism of aminoglycoside resistance by *Staphylococcus* spp. is enzymatic modification, in which modifying enzymes alter various sites on the aminoglycosides molecules so that the ability of drug to bind the ribosome and halt protein synthesis will be greatly diminished or lost[13]. Lindqvist and his colleagues, 2009 [14] showed that 9% were PVL-negative erythromycin, clindamycin and tobramycin (ECT) sensitive MSSA.

MRSA isolates results showed sensitive to ciprofloxacin, levofloxacin, and moxifloxacin. Mitscher, 2004 [15] has stated that ciprofloxacin is effective against S.aureus isolates, and this type of antibiotic inhibits bacterial growth by effecting DNA maintenance. Ciprofloxacin, is bacteriocidal drug, affecting Gram positive and Gram negative bacteria. Generally anti Gram negative activity is more closely associated with DNA gyrase inhibition, whereas anti Gram positive activity is more closely associated with bacterial topoisomerase IV inhibition. DNAgyrase, and bacterial topoisomerase IV, are vital for dicating the proper topology of DNA which is important for protein biosynthesis, DNA replication and DNA repair, this interference with DNA transcription, replication, and repair will promote its cleavage, leading to rapid bacterial cell death. Also resistance to ciprofloxacin is most commonly associated with genetic-based alterations in the topoisomerases, resulting in decreased drug binding.

The results revealed that bacterial isolates resistant to erythromycin. Macrolides inhibit protein synthesis by binding to the 50S ribosomal subunit causing an inhibition of translocation of peptidyl-tRNA and the initial steps of 50S subunit assembly. The spectrum of activity of macrolides includes aerobic Gram positive bacteria, including *Staphylococcus* spp. [16]. MRSA isolates results showed resistant to clindamycin. The most common mechanism for such resistance is target site modification mediated by *erm* genes, which can be expressed either constitutively or inducibly [17]. Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin-resistant and clindamycin sensitive *in vitro* when not placed adjacent to each other.

In this study, results found that bacterial isolates resistant to teicoplannin. Glycopeptide antibiotics are produced by actinomycetes and inhibit the synthesis of bacterial cell wall by blocking peptidoglycan assembly. They bind to the D-alanyl-D-alanine (D-Ala-D-Ala) C terminus of the nascent peptidoglycan and prevent it from being utilized in the following cross-linking reactions catalyzed by transglycosylases and transpeptidases [18].

MRSA isolates results revealed resistant to tetracycline but sensitive to tigecycline. Resistance to tetracyclines occurs by three mechanisms (efflux, ribosomal protection, and chemical modification). The first two mechanisms are the most important and occur as follows: efflux pumps elements, located in the cytoplasmic membrane, are responsible for pumping the drug out of the cell. TET protein is *tet* products which responsible for protecting the ribosome, likely through mechanisms that induce conformational change. These conformational changes either prevent binding of the tetracyclines or cause their dissociation from the ribosome. It is often plasmid-controlled [19]. The bacterial isolates results found that resistant to rifampicin. Rifampin acts by interacting specifically with the β subunit of the bacterial RNA polymerase encoded by the *rpo*B gene. Rifampin resistance in *Escherichia coli* and *S.aureus* is due to alterations in the target leading to a reduced affinity of the enzyme for the antibiotic. Fluit and his co-workers, 2001 [20] demonstrated that only 2% of the MSSA isolates were multidrug resistant (oxacillin, penicillin, gentamicin, erythromycin, clindamycin, ciprofloxacin, tetracycline, rifampin, and chloramphenicol), however, 87% of the MRSA isolates were multidrug resistant and only 3% of the MRSA isolates were resistant to β-lactam antibiotics only.

The present study results showed MRSA isolates sensitive to trimethoprim/sulfamethoxazole. Trimethoprim is a tetrahydrofolate

reductase inhibitor that, when added to sulfamethoxazole, provides a second step block in the folate biosynthetic pathway. TMP-SMZ proved to be bactericidal [21]. Blocking folate metabolism at 2 sites decreased the emergence of resistance, nevertheless, resistance to TMP-SMZ has occurred because of amino acid substitutions in both enzymes, Plasmids (e.g., pSK41) carry the altered genes, which facilitate the spread of TMP-SMZ resistance. Chen and his colleagues, 2006 [22] showed that 98% and 94% of CA-MRSA were susceptible to trimethoprim/sulfamethoxazole and clindamycin (confirmed by D test), respectively, however, Randrianirina and his co-workers, 2007 [23] found that susceptibility to TMP-SMZ were 91%.

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