

The Patterns Of Selected Antibiotics Sensitivity And Resistance To Staphylococcus Aureus Isolates

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Abstract: Complications of Staphylococcus aureus infection have greatly increased in recent past because of the many invasive procedures, increased cases of immunocompromised individuals, and the uprising trends in increased antimicrobial resistance of S. aureus strains. Despite of these available information and by contrast with developed countries, S. aureus associated disease are ranked low on the public-health agenda in Kenya and other developing countries. Therefore there is due reason to undertake an investigation and report the trends and patterns in a thorough manner, majorly and especially regarding the antimicrobial resistance. The aim of this study was thus to determine the levels of drug resistance of Staphylococcus aureus to various classes of antibiotics. This data is of significance in improving baseline data on antibiotic resistance of S. aureus isolated from human clinical specimens for the prudent use of antibiotics and the coming up with policies on control programs. All culture isolates were confirmed as Staphylococcus aureus genus by various tests, That is, gram staining, catalase and oxidase. Catalase positive, gram positive and oxidase negative isolates were defined as Staphylococcus. Further analyses by mannitol salt agar fermentation of the isolates and positive coagulase tests indicated Staphylococcus aureus. The area of clearance of sensitivity and tolerance was measured in millimeters and categorized as sensitive, resistant or intermediate. The present study reported that, S. aureus was most sensitive to Azithromycin, whereby 46 (61%) samples were sensitive. Penicillin on the hand was least sensitive showing 29% level of sensitivity. Methicillin, Gentamicin had more than 50% level of sensitivity, That is, 41 (55%) and 40 (53%) respectively. Other antibiotic drugs including ampicillin, augmentin and tetracycline demonstrated less than 50% sensitivity, That is, 29 (39%), 32 (43%) and 33 (44%) respectively. Drug resistance for S. aureus was therefore reported to be highest in penicillin (59%) and least in Azithromycin (25%). Based on results of this study we conclude that drug resistance of Staphylococcus aureus may vary with the antibiotics being used.

Index Terms: antibiotics, Azithromycin, Drug resistance; Drug sensitivity, Isolates, penicillin, Staphylococcus aureus

1 Introduction

Antibiotic resistance by Staphylococcus aureus (S. aureus) is a major public health threat. Though S. aureus is a normal flora on the skin and mucous membranes (Howden et al., 2011), it may cause a variety of infections, ranging from mild infections to serious post-surgical wound infections (Deurenberg & Stobberingh, 2008). Staphylococcus aureus has the ability to express different virulent factors and therefore it is taken as a medically important bacterium if detected in clinical specimens (Styers, Sheehan, Hogan, & Sahm, 2006). Complications of S. aureus infection have greatly increased in the recent past because of the many invasive procedures, increased cases of immunocompromised individuals arising particularly from HIV infection and cancer among other causes, and the uprising trends in increased antimicrobial resistance of S. aureus strains (Naber, 2009).

associated with the pathogen, is driving an urgent necessity for better strategies and monitoring for sensitive antibiotics for therapy and prevention of S. aureus infection. S. aureus is associated with hospitals and has now emerged as a common cause of community infection (Henry F Chambers & Deleo, 2009). The prescription of new antibiotics to manage this pathogen has frequently been followed by the uprising of resistant strains (Schito, 2006). Most significantly, S. aureus isolates resistant to beta-lactams have become common. Despite of these available information and by contrast with developed countries, S. aureus-associated diseases are ranked low on the public-health agenda in Kenya and other developing countries. Therefore there is due need to undertake an investigation and report on the trends and patterns in a thorough manner, majorly and especially regarding the antimicrobial resistance. The aim of this study was thus to determine the level of drug resistance of Staphylococcus aureus to various classes of antibiotics. This data is of significance in improving baseline data on antibiotic resistance of S. aureus isolated from human clinical specimens for the prudent use of antibiotics and the coming up with policies on control programs.

2 MATERIALS AND METHODS

In a cross-sectional study, 75 study patients were recruited, from both out-patient and in-patient departments of Moi Teaching and Referral Hospital (MTRH) as the study subjects. The study received an ethical approval from Moi Teaching and Referral Hospital (MTRH) institutional research ethical committee (IREC). MTRH is a national referral hospital which serves the larger western part of Kenya. Consecutive sampling technique was used to recruit study subjects until the required sample size for the study was achieved. Different anatomical sites were used for specimen collection. For patients who had respiratory infection, sputum specimen was collected; skin infection, pus swab was picked while in urinary tract infection urine specimen was collected. Isolation and identification of S.

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aureus were performed at the hospital's main microbiology laboratory. All the culture isolates were confirmed as *Staphylococcus aureus* genus by various tests, That is, gram staining, catalase and oxidase (Kateete et al., 2010). Catalase positive, gram positive and oxidase negative isolates were defined as *Staphylococcus*. Further analyses by mannitol salt agar fermentation of the isolates and positive coagulase tests indicated the presence of *Staphylococcus aureus*. In brief, after specimens were collected, samples from all specimens were inoculated in blood agar, Mackonkey agar (aerobically) and chocolate blood agar (anaerobically) overnight. Gram staining was then done from the three plates. If the colonies turned gram positive colonies, a catalase and oxidase tests were done followed by coagulase tests. Samples positive for coagulase test were then inoculated on Muller Hinton agar and a set of discs gram positive and broad spectrum drugs were incorporated and incubated at 37°C overnight. The isolated colonies were tested for their susceptibility to 7 selected common antimicrobial drugs namely ampicillin, methicillin, augmentin, penicillin, azithromycin tetracycline and gentamicin. The antibiotic susceptibility testing was done by disc diffusion method according to the guidelines provided by Clinical and Laboratory Standards Institute (Clsi, 2013). The area of clearance was measured in millimeters and categorized as sensitive, resistant or intermediate.

3 RESULTS AND DISCUSSION

The present study reported that, *S. aureus* was most sensitive to Azithromycin, whereby 46 (61%) samples were sensitive. On the hand, *S. aureus* was least sensitive to Penicillin showing 29% level of sensitivity. Methicillin, Gentamicin had more than 50% level of sensitivity, That is, 41 (55%) and 40 (53%) respectively. The susceptibility patterns of the tested antibiotics against *S. aureus* are shown in table 1.

Table 1: Susceptibility patterns of the tested antibiotics against *S. aureus*

	Sensitive		Resistant		Intermediate	
	n	%	n	(%)	n	%
Ampicillin	29	(39)	33	(44)	13	(17)
Methicillin	41	(55)	22	(29)	12	(16)
Gentamicin	40	(53.3)	28	(37.3)	7	(9.3)
Augmentin	32	(43)	21	(28)	22	(29)
Tetracyclin	33	(44)	28	(37)	14	(19)
Penicillin	22	(29)	44	(59)	9	(12)
Azythromycin	46	(61.3)	19	(25.3)	10	(13.3)

Other antibiotic drugs including ampicillin, augmentin and tetracycline demonstrated sensitivity less than 50%, That is, 29 (39%), 32 (43%) and 33 (44%) respectively. Drug resistance for *S. aureus* was therefore reported to be highest in penicillin (59%) and least in Azythromycin (25%). Intermediate sensitivities were observed in all antibiotics used and reported as shown in table 1 and figure 1.

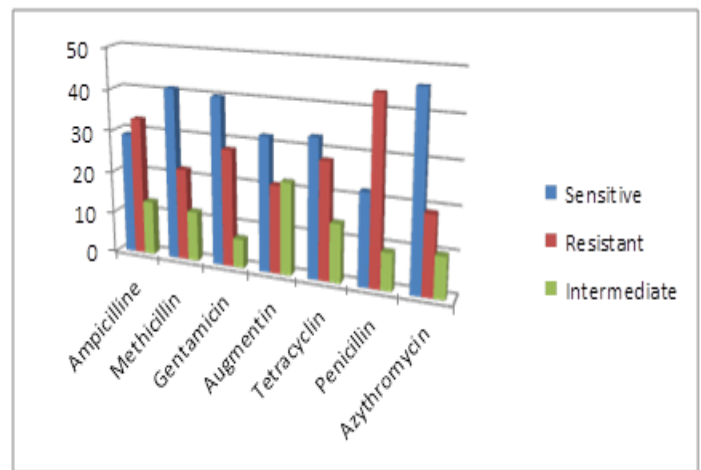


Figure 1: Susceptibility patterns of the tested antibiotics against *S. aureus* in numbers

Antibiotic resistance by antimicrobial-sensitive bacteria from resistant strains of bacteria happens through conjugation, transformation, or transduction. The use of antibiotics increases more pressure for the emergence of resistant strains (Tenover, 2006). In a similar study to the present, a methicillin resistance rate of 18.9 % was reported. In the same study 94 isolates were also observed to be gentamicin-resistant and 106 isolates to be resistant to tetracycline (Duran, Ozer, Duran, Onlen, & Demir, 2012). The study however observed that, generally the phenotypic antibiotic sensitivity patterns were not similar to those obtained by genotyping done by multiplex PCR. Styers et al in 2006 also observed that an increasing emergence of outpatient methicillin-resistant *S. aureus* exhibited multi-drug resistance (Styers et al., 2006). In another study within and between countries by den et al in 2013, it was reported that, the highest recorded resistance percent was to azithromycin (from 1.6% in Sweden to 16.9% in France) except for penicillin (den Heijer et al., 2013). Ninety one methicillin-resistant strains were also isolated, and the highest prevalence of this strain was reported in Belgium (2.1%). In concurrence with the present study a previous study observed that antibiotic resistance was mainly for penicillin G (Hoekstra & Paulton, 2002). Interestingly, closely after introduction of penicillin in 1940s, penicillin-resistant staphylococci were recognized, first in hospitals and subsequently in the community (Rammelkamp & Maxon, 1942). This explains that *S. aureus* has long history of antibiotic resistance. The present study results confirm that *S. aureus* has developed resistance for penicillin greatly showing the highest resistance of 59%. These patterns of antibiotic resistance as reported with penicillin that first emerge in hospital settings and then quickly spread to the community are now known to recur with each new wave of antimicrobial resistance (H. F. Chambers, 2001). More than 90% of staphylococcal isolates now produce penicillinase, regardless of the clinical setting (Lowy, 2003). Penicillin antibiotic resistance mainly spread as a result of the spread of the resistant strains *S. aureus*. *S. aureus* antibiotic resistance to penicillin was also reported to happen because of blaZ, a gene that codes for β -lactamase (Lowy, 2003). This enzyme was produced when staphylococci were exposed to β -lactam antibiotics, whereby the β -lactam rings are hydrolyzed, rendering it inactive. The present study, and contrary to many

studies, observed a sensitivity level of 55% compared to 29% of methicillin resistance by *S. aureus*. Among all antibiotics the quick spread of the methicillin-resistant strains has become a concern by medics. The outcome of treatment of diseases that are caused by methicillin-resistant *S. aureus* (MRSA) is worse compared to infections by methicillin-sensitive strains (Cosgrove et al., 2003). These observations can be as a result of underlying medical problems of patients infected by methicillin-resistant strains of *S. aureus* and the use of less effective antibiotics in the treatment of these infections, rather than to virulence factor of the methicillin-resistant strains. If identified in a community, these methicillin-resistant strains become the resident strains and account for the majority of nosocomial infections (Couto et al., 1995; Panlilio et al., 1992). Methicillin resistance by *S. aureus* is mainly because of *mecA* gene (H F Chambers, 1997). *MecA* produces penicillin-binding protein 2a an enzyme that enhances the trans-peptidation, important for cross-linkage of peptidoglycan chains (Ghuysen, 1994). Thus, *S. aureus* resistance to methicillin may also confer resistance to all β -lactam antibiotics. According to the present findings, gentamicin presents a better drug for *S. aureus* treatment in the current setting with sensitivity rate of 53.3 percent and hence remains a favorable drug among the aminoglycosides group of antibiotics. Data on the resistance for gentamicin by *S. aureus* remain scanty and therefore it could give an upper hand for treatment of *S. aureus* infections in the current setting. However, management of *S. aureus* infections can best be possible by use of more advanced and quick methods of diagnosis for detection of *S. aureus*, isolation and treatment of infected patients with effective antibiotics. Normal residence of *S. aureus* on human body increases the chance of subsequent infection, thus energy must be redirected to effective use of sensitive drugs against *S. aureus* for total elimination. In the recent past, the potential use of endopeptidase, lysostaphin, or phage lytic enzymes has also been considered for this purpose (Climo, Patron, Goldstein, & Archer, 1998; Peacock, De Silva, & Lowy, 2001). An antibiotic medication is easily and widely available from chemists in Kenya, where self-medication is likely the main driver for the emergence of antibiotic resistant strains of *S. aureus*. An initiative is needed to increase the understanding of *S. aureus* infections in Kenya and other developing countries to find practical solutions to the antibiotic resistance challenges posed by this now important universal pathogen (Nickerson, West, Day, & Peacock, 2009). Because of the changing pattern of antibiotic resistance in *S. aureus*, it would be of paramount importance to have a periodical monitoring of the changing patterns of drug resistance. New approaches to treatment and prevention should be of greater importance especially because of the diminishing availability and innovation of new effective antibiotics.

4 CONCLUSION

Based on results of this study we conclude that drug resistance of *Staphylococcus aureus* may vary with the antibiotics being used. The surveillance of antibiotic resistance and the spreading of *staphylococcus aureus* pathogen are of crucial importance in both hospital and community setups. We therefore recommend that *S. aureus* vaccine and further investigations to elucidate the cause of the antibiotic resistance should be the key focus.

5 Acknowledgments

We thank staff of MTRH and the institution management for allowing us to use their facilities. This research was also funded, in part, by MTRH. Appreciation also goes to the study participants who consented to give samples for this study. Finally, appreciations go to those entire not mentioned but directly or indirectly contributed to the success of this study.

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