Antibacterial activity of penicillins alone and in combination with different agents against *Staphylococcus aureus*

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Abstract: Difficulties in the treatment of the resistant strains of *Staphylococcus aureus*, which is a frequent cause of nosocomial infections in paediatric patients, has prompted this research to empower the usage of various combinations of penicillin. During the study period 17,452 clinical samples were processed for culture. The positive cultures yielded 564 strains of *S. aureus*. Out of these, 362 (64.2%) isolates were found to be methicillin sensitive *S. aureus* (MSSA) and 202 (35.8%) methicillin resistant *S. aureus* (MRSA). The frequency of *S. aureus* isolates from male patients (355; 63.1%) was found to be higher than female patients (209; 36.9%) and those from indoor wards (441; 78.2%) were more than the outdoor wards (123; 21.8%). Frequency distribution of *S. aureus* showed to be highest among blood 342 (60.6%) and cerebrospinal fluid 100 (17.8%) samples. The sensitivity pattern of MSSA with piperacillin-tazobactam was 344 (95.0%), ampicillin-sulbactam 340 (93.9%), co-amoxiclav 332 (91.8%) and ampicillin-oxacillin 257 (71.0%). MRSA susceptibility to piperacillin-tazobactam was 143 (71.0%), ampicillin-sulbactam 114 (56.6%), co-amoxiclav 61 (30.2%) and ampicillin-cloxacillin 18 (9%). The Cochran Mantel Haenszel test showed that the effectiveness for each penicillin was associated significantly ($p<0.05$) with both the MSSA and MRSA. The combinations of piperacillin-tazobactam, ampicillin-sulbactam, co-amoxiclav and ampicillin-cloxacillin exhibited higher efficacy than using them alone to combat *Staphylococcal* infections.

Keywords: *Staphylococcus aureus*, penicillin, combinations of penicillin, antimicrobial activity.

INTRODUCTION

S. aureus is a non-spore former and non-motile Gram positive bacteria discovered in 1880 from surgical abscesses (Lowy, 1998). *S. aureus* expresses many potential virulence factors such as Protein A, enzymes, toxins and may also have inherent and acquired resistance to antibiotics (Verkaik *et al*., 2010). In past few years an increase in prevalence of *Staphylococcal* infection has been observed in varies parts of the world (Chambers, 2001). *S. aureus* is the most common nosocomial pathogen and is responsible for many diseases such as cellulitis, impetigo, furuncles, carbuncles, bacteremia, septicemia, abscesses, meningitis, joint infections and endocarditis (Cimolai *et al.,* 2008). *S. aureus* is also associated with high mortality rate among children causing severe diseases like scalded skin syndrome and multiple brain abscesses. *S. aureus* dissemination is commonly expedited because of indigent infection control measures, prolonged duration of hospital stay, injudicious use of antibiotics and unhygienic procedures (Woodlief *et al*., 2009). Methicillin resistant *S. aureus* (MRSA) are resistant to the effect of methicillin and similar drugs such as penicillin G and amoxicillin (Qin *et al.,* 2014; Tan *et al.,* 2015).

Penicillin was discovered from the molds of *P. chrysogenum* and *P. notatum* by Nobel laureate Sir Alexander Fleming (Fleming, 1929). Penicillin has a penam structure having the formula $R\text{-}C_9H_{11}N_2O_4S$. The "R" is a functional side chain which inhibits the cross linking of peptidoglycans in cell wall of Gram positive bacteria. The enzymes hydrolyzing this cross linkage of peptidoglycan resulting in cell death (Fisher *et al.,* 2005). The four classes of penicillin include the penicillin which are resistant to penicillinase (oxacillin and methicillin), natural penicillin (penicillin G), amino-penicillin (amoxicillin and ampicillin) and piperacillin which is an expanded spectrum penicillin (Dalhoff *et al.,* 2006). Resistance among *S. aureus* strains has raised due to the formation of β-lactamase enzymes in the bacteria which palliates the effect of β-lactam antibiotics (Korzeniowski *et al.,* 1982).

The objective of our study was to evaluate the occurrence of *S. aureus* and comparison of antibacterial activity of selective penicillin alone and in combination with different agents including other penicillins (piperacillin, cloxacillin) and beta-lactamase inhibitors (clavulanic acid and sulbactam). Morbidity and mortality among the paediatric patients infected with *S. aureus* can be decreased significantly with early detection and appropriate management with penicillin combinations.

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MATERIALS AND METHODS

Materials

The materials used in the study included Gram's stain, hydrogen peroxide, DNase agar, Blood agar, MacConkey agar, Mueller-Hinton agar and Coagulase test kit. Various antimicrobial discs of piperacillin-tazobactam (100/10µg), ampicillin-sulbactam (10/10µg), co-amoxiclav (20/10 µg), ampicillin-cloxacillin (10µg), ampicillin (10µg), cloxacillin (5µg), amoxicillin (25µg), penicillin G (10 U) and methicillin (10µg) were used to observe the antibacterial activity of penicillin. All culture media and antibiotic discs used during the study were obtained from Oxoid UK.

Collection of clinical samples

The present study was conducted at The Children's Hospital & the Institute of Child Health Lahore, Pakistan, from April to September in 2011.Various clinical specimens such as blood, cerebrospinal fluid, pus, ear and nasal swabs were collected from both genders in the age group from newborn to fifteen years. The specimen were collected consecutively and non-repetitively. The blood specimens were inoculated in Brain Heart Infusion Broth soon after collection.

Specimen processing

The specimens were inoculated on Blood, Chocolate and MacConkey agar plates (90mm). The plates were incubated at 35ºC for 18-24 hours in anaerobic incubator. Identification was performed based on bacterial morphology, Gram's stain reaction, DNase, catalase and coagulase tests. All the tests were performed according to description in Cheesbrough (Cheesbrough, 2006). *S. aureus* (ATCC 25923) used as reference strain.

Antimicrobial sensitivity testing

In vitro antimicrobial susceptibility testing of the isolates was performed using Kirby Bauer Disc diffusion method. A homogenous suspension of the bacteria was prepared and compared to McFarland 0.5 turbidity standard. A sterile swab soaked with the bacterial suspension was used to make an even lawn of bacteria on the Mueller-Hinton agar plates. Various antibiotic discs of penicillins alone and in combination with other agents were applied at about 20 mm distance from each other using a disc dispenser. The plates were incubated aerobically at 37ºC for 18-24 hours. The interpretation of antibiotics zones was made according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2011).

STATISTICAL ANALYSES

Data was analyzed using the statistical program SPSS v16.0 for Windows. A Chi square test for independence was employed for analysis. The statistical differences were determined using the Cochran Mantel Haenszel test. The results with $p<0.05$ were considered as significant.

RESULTS

A total number of 564 *S. aureus* were isolated throughout the duration of study. The MSSA strains were 362 (64.2%) while 202 (35.8%) were MRSA. The highest frequency of *S. aureus* was found in blood samples 342 (60.6%) followed by cerebrospinal 100 (17.8%), wound swabs 61 (10.8%), urine 34 (6%) and peritoneal dialysis catheters 10 (1.7%). *S. aureus* were less frequently isolated from other specimens. The frequency of *S. aureus* isolates from male and female patients was 355 (63.1%) and 209 (36.9%), respectively. There were 441 (78.2%) cases isolated from in door and 123 (21.8%) from outdoor patients (table 1). table 2 shows statistically significant relationship between type of *S. aureus* (MRSA/MSSA) and gender of patient from which it was isolated; however it shows association neither with type of wards from which isolates were collected (indoor/outdoor wards) nor with type of specimen.

The MSSA showed susceptibility to piperacillin-tazobactam 344 (95%), ampicillin-sulbactam 340 (93.9%), co-amoxiclav 332 (91.8%) and ampicillin-cloxacillin 257 (71%). MSSA showed lesser sensitivity to cloxacillin 172 (47.4%), ampicillin 120 (33.0%), amoxicillin 116 (32.1%) and penicillin G 72 (20.0%). The MRSA strains were susceptible to piperacillin-tazobactam 143 (71%), ampicillin-sulbactam 114 (56.6%), co-amoxiclav 61 (30.2%) and ampicillin-cloxacillin 18 (9%) whilst none of the MRSA strain was susceptible to penicillin when applied without combination (table 3).

The Cochran Mantel Haenszel test allowed the comparison of two groups; MRSA and MSSA on a categorical response of effectiveness of selected penicillin and showed that the effectiveness for each penicillin was associated significantly ($p<0.05$) with both the MSSA and MRSA (table 4).

DISCUSSION

S. aureus is a significant clinical pathogen that causes life threatening nosocomial and catheter related infections especially among the paediatric patients (Tan *et al.*, 2015). This work has documented the recent data about the frequency distribution and *in vitro* susceptibility pattern of *S. aureus* isolated from various paediatric clinical samples against selected penicillins alone and in combination with different agents. A study reported higher prevalence of *S. aureus* infections in males (58.9%) compared to females (41.1%) in intensive care units (Jiang et *al.,* 2004). This finding correlates with the result of current study in which frequency of *S. aureus* infection in males (63.1%) is higher than in females (36.9%). Another study in Imam Komeini Hospital Urmia, Iran, determined a proportion of male to female patients infected with *S. aureus* to be 1.67:1 that is also in accordance with the findings of our study (Shahsanam *et al.*, 2008).

Table 1: Demographic distribution of *S. aureus* (n=564)

Characteristics	Number	Percentage
Type of specimen		
Blood	342	60.6%
Cerebrospinal fluid	100	17.8%
Wound swab	61	10.8%
Urine	34	6.0%
Peritoneal dialysis catheter	10	1.7%
Endo tracheal tube	7	1.3%
Central venous puncture tip	6	1.0%
Tracheal secretions	4	0.7%
Gender distribution of *S. aureus*		
Males	355	63.1%
Females	209	36.9%
Ward distribution of *S. aureus*		
In-patients	441	78.2%
Out-patients	123	21.8%

Table 2: Chi Square test of association of type of *S. aureus* with various factors.

Variables	Pearson's Chi square	Degree of freedom	p-value
Type of *S. aureus*-Gender	64.44	1	0.002
Type of *S. aureus*-Wards	0.001	1	0.10
Type of *S. aureus*-Specimen	0.461	7	0.21

Table 3: Comparison of sensitivity pattern of penicillins alone and its various combinations on MSSA (n=362) and MRSA (n= 202)

Antibacterial Agents	Sensitive		Intermediate		Resistant	
	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA
Piperacillin-tazobactam	344 (95%)	143 (71%)	11(3)	22 (11%)	7 (2%)	37 (18%)
Ampicillin-sulbactam	340(93.9%)	114(56.6%)	12(3.3%)	49(24.1%)	10(2.8%)	39(19.3%)
Co-amoxiclav	332(91.8%)	61(30.2%)	21(5.7%)	26(12.8%)	9(2.5%)	115(57%)
Ampicillin-cloxacillin	257(71%)	18(9%)	72(20%)	4(2%)	33(9%)	180(89%)
Cloxacillin	172(47.4%)	0(0%)	144(40%)	42(21%)	46(12.5%)	160(79%)
Ampicillin	120(33%)	0(0%)	137(38%)	54(26.6%)	105(29.1%)	148(73.4%)
Amoxicillin	116(32.1%)	0(0%)	8(2.3%)	39(19.4%)	238(65.6%)	163(80.6%)
Penicillin G	72(20.0%)	0(0%)	100(27.5%)	56(27.5%)	190(52.5%)	146(72.5%)
Methicillin	362(100%)	0(0%)	0(0%)	0(0%)	0(0%)	202(100%)

Table 4: Cochran Mantel Haenszel test

Antibiotics	Pearson's Chi-Square	Degree of freedom	Asymp. Sig. (2-sided)(p-value)
Piperacillin-tazobactam	67.089	2	.000
Ampicillin-sulbactam	116.058	2	.000
Co-amoxiclav	252.988	2	.000
Ampicillin-cloxacillin	353.027	2	.000
Cloxacillin	267.131	2	.000
Ampicillin	128.313	2	.000
Amoxicillin	114.281	2	.000
Penicillin G	48.702	2	.000
Methicillin	564.00	1	.000

In the present study majority of *S. aureus* were isolated from bacteremia patients (60.6%) followed by cerebrospinal fluid (17.8%), wound swab (10.8%) and urine (6.0%). These results are also supported by other studies. A work conducted at Diskapi Hospital, Ankara, Turkey reported *S. aureus* 114 (46.2%) from blood samples, 51 (20.7%) wound swabs and 48 (19.4%) urine samples (Annie *et al.*, 2002). Similar study on the occurrence of *S. aureus* at National Institute of Health Islamabad, also revealed that maximum isolates were from blood followed by wound and ear swabs (Siddiqi *et al.,* 2002).

Present data shows that *S. aureus* infection predominates in inpatients (78.24%) as compared to outpatients (21.76%), maybe due to cross infections transmitted by contaminated equipment or procedures leading to hospital acquired infections. This situation leads to high morbidity, prolonged hospital stays, increased treatment cost and thus presents a major challenge for clinical governance. A relevant study on 108 *Staphylococci*, obtained from indoor air samples of the hospital revealed that most of these strains were capable to produce biofilms and showed multi-resistance profiles. This also clarified the significance of hospital air as means of dissemination of *Staphylococci* (Botelho *et al*., 2012).

In our study, 69.2% *S. aureus* were MSSA and 30.8% were MRSA. A study conducted at Medical Centre Karachi, Pakistan reported 42% incidence of MRSA from eight different laboratories all over the country (Hafiz *et al.,* 2002). However, Theresa *et al.,* (2005) reported lesser prevalence of MRSA from countries including France (6%), Ireland (5%) and United Kingdom (2%). This is obvious that MRSA prevalence is much higher in developing countries as compared to developed countries.

In the current study a significantly higher sensitivity was seen with combination of ampicillin and sulbactam against both MSSA (93.9%) and MRSA (56.6%) in comparison with ampicillin alone against MSSA (33%) and MRSA (0%). These results are in accordance with a study, which expressed a higher effectiveness of ampicillin-sulbactam in comparison to ampicillin alone against the strains isolated from various clinical specimens (Bush, 1998).

Similarly the combination of co-amoxiclav showed a higher sensitivity against MSSA (91.8%) and MRSA (30.2%) than with amoxicillin alone against MSSA (32.1%) and MRSA (0%). A related study demonstrated a relatively higher effectiveness of amoxicillin-clavulanic acid (90%) than amoxicillin alone (38%) against *S. aureus* collected from paediatric patients (Ahmed *et al.,* 2002).

The dilemma of growing resistance remains a continuing threat for the developing countries due to higher rates of antibiotic resistance gene transfer (World Health Organization, 2000). The emergence of resistant strains of *S. aureus* can be reduced by the implementation of good infection control policy, prompt diagnosis and timely reporting of *in vitro* antibiotic sensitivity. The selection of appropriate combinations of antibiotics can also help to reduce the side effects and unnecessary increase in financial burden. In this context, the present study determined the frequency and antibiotic susceptibility profile of *S. aureus,* which will be helpful in formulating appropriate antibiotic policies to control nosocomial infections.

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