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Antibiotic Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* from Septicemia Suspected Children in Tertiary Hospital in Hosur, South India

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Abstract Septicemia is an important cause of morbidity and mortality among children in India. A wide variety of bacterial and fungal pathogen can cause septicemia. The objective of the present study is to determine the antibiotic susceptibility pattern of methicillin resistance *Staphylococcus aureus* from septicemia suspected children. A cross-sectional study was undertaken from June, 2011 to August, 2012. A total of 300 children were screened. Study subjects were conveniently selected until the required sample size is full filled; blood samples were collected, transported and microbiologically processed using standard procedures; and data was cleaned and entered into a computer and statistical analysis was performed using SPSS for windows version 16. 88 (29.3%) were culture positive. *S. aureus* was the prominent isolate 26 (29.54 %). Of which 8(30.76% were methicillin resistant (MRSA). The present study reveals MRSA isolates were resistant to mostly all antibiotics and were sensitive to oxacillin and vancomycin.

Keywords: *MRSA, antibiotic, susceptibility pattern, septicemia, children*

1. Introduction

Staphylococcus aureus is a frequent cause of infections in children, ranging from skin and soft tissue to invasive life-threatening infection [1]. It is an important cause of community and hospital infections [2]. Community-associated MRSA (CA-MRSA) infection in children is an increasing public health problem [3]. These CA-MRSA strains cause serious skin and soft tissue infections, necrotizing pneumonia, and sepsis in healthy children [4].

Methicillin Resistant *Staphylococcus aureus* (MRSA) has been the most commonly recognized multidrug-resistant pathogen in the universe and the emergence of MRSA strains found in increasing number of infections and often multi drug resistant in nature now pose serious therapeutic problems to clinicians [5,6]. MRSA strains are usually resistant to several groups of broad spectrum antibiotics that are used on a large scale in the hospital. The mechanism of increased spreading under antibiotic pressure may have contributed to the worldwide increases in the prevalence of MRSA in hospitals [7,8]. The most remarkable feature of *S. aureus* is its ability to acquire resistance to antibiotics. Resistance to penicillin is mediated by the production of β-lactamase. Many resistance genes are acquired by plasmid mediated gene transfer and some may be transferred to the chromosome as mobile genetic elements. Methicillin resistant strains usually possess more than four genes encoding different resistant mechanism [8]. Therefore, treatment of MRSA infections is challenging and empiric treatment usually includes the use of clindamycin or vancomycin. MRSA strains that are clindamycin-susceptible but erythromycin-resistant may have the in vitro inducible macrolide, lincosamide and streptogramin B (MLSB)-resistance phenotype with potential for treatment failure. Rates of inducible MLSB resistance among pediatric MRSA isolates vary widely [3].

One study in South Africa indicated antibiotic resistance of MRSA to erythromycin, tetracycline, trimethoprim/sulfamethoxazole, gentamicin and ciprofloxacin ranged between 55% and 78%, while all isolates were susceptible to teicoplanin, linezolid, vancomycin and quinopristin/dalfopristin [6]. Similar study in India revealed Methicillin resistance among the *Staphylococcus aureus* isolates was 39.5% and resistance to all antibiotics tested among the Methicillin-resistance and Methicillin-sensitive-*staphylococci* was found to be 26.3% and 6.8% respectively [7].

There is a growing concern about the rapid rise in resistance of *S. aureus* to antimicrobial agents. Trends in the antibiotic susceptibility of MRSA are regularly investigated in many countries, but minimal countrywide data are available for India particularly for in the study area. Methicillin-resistance is a useful marker in selecting appropriate antimicrobial agents for treatment of infections caused by *S. aureus* changing pattern of resistance of *S. aureus* makes its periodic surveillance mandatory [7]. The present study was focused to

determine the antimicrobial susceptibility pattern of MRSA from septicemia suspected children.

2. Materials and Methods

2.1. Blood Culture Method

The blood sample for culture was obtained from children having a clinical picture suggestive of septicemia/bacteremia before instituting antibiotic therapy. Total 300 blood specimens were collected using aseptic techniques. 2 to 5ml blood was drawn from each patient and was distributed in to brain heart infusion (BHI) blood culture bottles (Himedia, India). The BHI blood culture bottles were incubated at 37 ℃ for aerobic and facultatively anaerobic environment for 7 days. The culture bottles were discarded if there was no growth after 7 days incubation. Any blood culture bottles flagging positive was gram stained and sub cultured on to selective and non-selective media such as Nutrient agar, Blood agar, Mannitol salt agar and Staphylococcus agar. The isolated pathogens from the blood culture were identified by standard biochemical identification methods. Yellow colonies grew on manitol salt agar and golden yellow colonies appeared on nutrient agar showing gram-positive grape-like coccoid clusters and producing DNase and coagulase were identified as *S. aureus*. The total numbers of positive culture bottle within the sets were determined.

2.2. Antibiotic Susceptibility Testing

The antibiotic susceptibility pattern of all the staphylococcal strains was determined by modified Kirby Bauer disc diffusion method against the following antibiotics: chloramphenicol (30 µg), penicillin (60 µg), Amoxicillin (25 µg), oxacillin (1 µg), erythromycin (15 µg), gentamicin (10 µg), *Trimethoprim-sulfamethoxazole* (1.25ug/23.75ug), ciprofloxacin (5 µg), Rifampicin (30 µg), erythromycin (15 µg), vancomycin (30 µg) and methicillin (5 µg) (Himedia, India). All tests were performed on Muller-Hinton agar, and were interpreted after incubation for 24 h at 37 ℃. The zone diameters measured around each disk were interpreted on the basis the Clinical and Laboratory Standards Institute (CLSI) [9]. *S. aureus* ATCC 25923 was used as a standard control strain.

2.3. Screening Test for MRSA

Screening test was performed in accordance to the CLSI guidelines using oxacillin agar [9]. Briefly, a bacterial suspension of 10^6cfu/ml was prepared from each isolate. Then a swab was dipped and streaked on the surface of a Muller-Hinton agar supplemented with 6 µg/ml oxacillin and 4% NaCl. After incubation for 24hrs at 35 ℃, if any growth was detected, the isolate was considered MRSA.

3. Results

The present study identified 88 septicemia children out of 300 suspected children screened (29.33%). The predominant isolate was *Staphylococcus aureus* which accounted 29.54% of the isolates. MRSA accounted 30.76% of *S. aureus* isolates (Table 1).

Table 1. Proportion of Methicillin Resistance *S. aureus* (MRSA) with respect to Sex in Septicaemia suspected Children in Hosur, South India (n= 26)

	MRSA		Methicillin Sensitive *S. Aureus*		Total	
Sex	No.	%	No.	%	No.	%
Male	5	19.2	10	38.5	15	57.7
Female	3	11.5	8	30.8	11	42.3
Total	8	30.7	18	69.3	26	100

The antimicrobial susceptibility pattern of MRSA isolates against agents of different classes is summarized in Table 2. The drug resistance patterns of MRSA isolated from blood samples was found to be highly variable. All the MRSA strains were resistance against penicillin, rifampicin, amoxicillin, gentamicin, methicillin and all are susceptible to vancomycin and oxacillin.

Table 2. Antimicrobial Susceptibility Pattern of Methicillin Resistance Staphylococcus aureus (MRSA) from Septicaemia suspected Children in Hosur, South India

Antibiotic	Methicillin Resistance *Staphylococcus aureus* (MRSA) (n= 8)		
	S [n (%)]	I [n (%)]	R [n (%)]
Am	ND	ND	8 (100)
ERT	2 (25)	3 (37.5)	3 (37.5)
SXT	3 (37.5)	ND	5 (62.5)
CIP	5 (62.5)	2 (25.0)	1 (12.5)
A	ND	ND	8 (100)
G	ND	ND	8 (100)
C	6 (75.0)	ND	2 (25.0)
Ox	8 (100)	ND	ND
P	ND	ND	8 (100)
R	ND	ND	8 (100)
Va	6 (75.0)	2 (25)	ND
M	ND	ND	8 (100)

MRSA= Methicillin Resistance *Staphylococcus aureus* , ND= Not detected, AM= Amoxacillin, A= Ampicillin, G= Gentamycin, Ox= Oxacillin, P= Penicillin-G, R= Rifampicin, Va= Vancomycin, M= Methicillin, C= Chloramphenicol, ERT= Erythromycin, SXT= Trimethoprim sulfamethoxazole, CIP= Ciprofloxacin, S= Susceptible, I= Intermediate, R= Resistance

4. Discussion

The septicemia infection is prevalent children in India, especially the age group between 1-5 years. The prevalence rate of MRSA infection in this study was found to be 30.7% which is corroborate with studies conducted in Nepal [10] and India [11,12,13]. There is a growing concern about the rapid rise in resistance of *S. aureus* to antimicrobial agents [14]. In this study, all clinical MRSA strains (100%) were resistant to penicillin, rifampicin, ampicillin, gentamicin, and amoxacillin. A similar result was noted for gentamicin and penicillin in South India [15,16] and Tridad and Tobago (Southern Caribbean) [17].

To our knowledge, the present study provides the most comprehensive description to the epidemiology and burden of MRSA infection among Septicemia Suspected children in Hosur, South India. We found that more than half of MRSA isolates were susceptible to oxacillin (100%), vancomycin (75%), chloramphenicol (75%), and

ciprofloxacin (62.5%). A similar result was reported to ciprofloxacin and vancomycin from South Africa [6], Tridad and Tobago [17], and Pakistan [18]. During the study period, we identified a stable increase in both the overall incidence of *S. aureus* infection and the incidence of MRSA infection

5. Conclusion

The present study identified a recent increase in MRSA infection among septicemia suspected children. The regular monitoring antibiotic susceptibility pattern of MRSA and selection of a definite antimicrobial agent may be helpful for reducing the incidence of MRSA infections in septicemia children. These measures need to be implemented consistently in order to reduce the burden of MRSA infection in the hospital environment.

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Statement of Competing Interests

The authors declare no potential conflicts of interest.

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