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Incidence of methicillin resistant *Staphylococcus aureus* (MRSA) from septicemia suspected children

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Abstract: The objective of the present study is to determine the incidence, clinical features, bacteriological pattern and antibiotic sensitivity profile of septicemia suspected children in Tamil nadu, India. This study was undertaken in SBS Hospital Pvt. Ltd., Hosur and SRM Medical College and Hospital, Chennai. All children (Age 1-5 yr) admitted with clinical features and risk factors, suspected with septicemia were selected and the blood samples were collected. The study identified 54 septicemia or bacteremia children out of 298 suspected children screened (18.12 %). The isolated organisms from the blood sample were characterized by primary, biochemical and selective media identification methods. From the identified isolates, *Staphylococcus aureus* was found to be prominent organism (48.14 %). Then the screening of MRSA was carried out using standard method (38.46%). The antibiotic sensitivity pattern of *S. aureus* differs widely between methicillin resistant and sensitive isolates. The present study reveals that in case of MRSA isolates resistant to nearly all antibiotics, they were sensitive to oxacillin and vancomycin.

Keywords: Septicemia, MRSA, antibiotic resistance, clinical study, epidemiology, child health, India,

Introduction

Staphylococcus aureus is a facultatively anaerobic, Gram-positive coccus, which appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates (Ryan & Ray, 2004). *S. aureus* is catalase positive and able to convert hydrogen peroxide (H_2O_2) to water and oxygen, which makes the catalase test useful to distinguish staphylococci from enterococci and streptococci. *S. aureus* generally produces "coagulase", an enzyme that causes clot formation while most other *Staphylococcus* species are coagulase-negative (Ryan & Ray, 2004). Though majority of *S. aureus* are coagulase-positive, some may be atypical in that they do not produce coagulase. Incorrect identification of an isolate can impact implementation of effective treatment (Matthews *et al.*, 1997).

A wide variety of bacterial and fungal pathogen can cause septicemia, the clinical name for blood poisoning. One of the major pathogens causing septicemia is *Staphylococcus* spp., particularly methicillin resistant *S. aureus* (MRSA). It can enter the normally sterile blood stream either from a local site of infection (wound, ulcer, and abscess). The symptoms are not specific to MRSA and can be the same for other bacteria that cause septicaemia. Typical symptom includes high fever, raised white cell count, rigor (shaking), disturbance of blood clotting with a tendency to bleed and failure of vital organs. This is the kind of MRSA infection that has the highest death rate.

Antibiotic resistance in *S. aureus* was almost unknown when penicillin was first introduced in 1943 by Alexander Fleming who observed the antibacterial activity of the penicillium mould against a culture of *S. aureus*. In 1950, 40% of hospital *S. aureus* isolates was penicillin resistant; and by 1960, this had risen to 80% (Chambers, 2001). Staphylococcal resistance to penicillin is mediated by penicillinase or a form of β-lactamase production. Penicillinase-resistant penicillins such as methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin are able to resist degradation by staphylococcal penicillinase. The mechanism of resistance to methicillin is mediated via the *mec* operon, part of the staphylococcal cassette chromosome mec (SCC*mec*). Resistance is conferred by the *mecA* gene, which codes for an altered penicillin-binding protein (PBP2a or PBP2') that has a lower affinity for binding β-lactams (penicillins, cephalosporins and carbapenems).

S. aureus has become resistant to many commonly used antibiotics. In the UK, only 2% of all *S. aureus* isolates are sensitive to penicillin with a similar picture in the rest of the world, due to a penicillinase (a form of β-lactamase). The β-lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin and flucloxacillin) were developed to treat penicillin-resistant *S. aureus* and are still used as first-line treatment. In 1959, methicillin was introduced for the treatment of *S.aureus* infection, but only two years later, the first case of methicillin-resistant *S. aureus* (MRSA) was reported in England (Jevons, 1961). Despite this, MRSA generally remained an uncommon finding even in hospital settings until the 1990s when there was an explosion in MRSA prevalence in hospitals where it is now endemic (Johnson *et al.*, 2001).

The number of MRSA infections in the United States has increased significantly. A 2007 report in Emerging Infectious Diseases, a publication of the Centers for Disease Control and Prevention (CDC), estimated that the number of MRSA infections in hospitals doubled nationwide, from ~127,000 in 1999 to 278,000 in 2005, while at the same time deaths increased from 11,000 to more than 17,000 (Klein *et al.*, 2005). Another study led

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by the CDC estimated that MRSA would have been responsible for 94,360 serious infections and associated with 18,650 hospital stay-related deaths in the United States in 2005 (Kelvens *et al*., 2007). These figures suggest that MRSA infections are responsible for more deaths in the U.S. each year than AIDS (Stein *et al*., 2007). It has been argued that the observed increased mortality among MRSA-infected patients may be the result of the increased underlying morbidity of these patients. Several studies were found MRSA bacteremia to have a higher attributable mortality than methicillin-susceptible *S. aureus* (MSSA) bacteremia (Blot *et al*., 2002). A population-based study of the incidence of MRSA infections in San Francisco during 2004-5 demonstrated that nearly 1 in 300 residents suffered from such an infection in the course of a year and that greater than 85% of these infections occurred outside of the health care setting (Liu *et al*., 2008). MRSA now represents global problem, some large outbreaks reported from different parts of the world, where it had caused severe infections including septicemia, endocarditis and meningitis (WHO, 1996). The present work focused on to determine the Incidence of MRSA from septicemia suspected children and their antimicrobial sensitivity pattern.

Materials and methods

Media and chemicals

All media and chemical components purchased from Hi-media laboratories pvt. Ltd. (Mumbai, India) and Sigma Chemicals (St.Louis, USA).

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 298 blood specimens were collected by nurses using aseptic techniques during the study period of one year June 2006 to June 2007 from SBS Hospital Pvt. Ltd., Hosur and SRM Medical College and Hospital in Chennai. Skin antisepsis was performed by application of 70% isopropyl alcohol, followed by the application of 10% povidone-iodine. The skin was allowed to dry for 30-60 sec, and then 2 to 5ml blood was drawn from each patient and distributed brain heart infusion blood culture bottles (Hi Media Pvt. Ltd., India). The BHI blood culture bottles were incubated at 37^0C for aerobic and facultatively anaerobic environment for 7 days. The culture bottles were discarded if there was no growth. Any blood culture bottles flagging positive was gram stained and sub culture 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 primary, biochemical and selective media identification methods. The total numbers of positive culture bottle within the sets were determined.

Screening of MRSA from blood culture isolates

A total of 26 isolates of *S. aureus* were made during the study period (June 2006 to June 2007). Standard isolation procedure applied to all the blood samples. The blood culture isolates of *S. aureus* were tested for methicillin resistant using susceptibility test (1µgm/disc from Hi-media laboratories Pvt., Ltd., India) was performed by Kirby-Bauer's disc diffusion on Muller Hinton agar with 24 hours incubation at 35^oC. The result was interpreted according to the recommendation of the National Committee for Clinical Laboratory Standards (NCCLS, 1997). Zone of inhibition of less than 10 mm or any discernible growth with in the zone of inhibition was taken as indicative of methicillin resistance (screening out MRSA strain). The methicillin resistance was conformed by agar screen test using Muller Hinton agar plate supplemented with 4% NaCl and methicillin (5 µg/disc) (Jamal *et al*., 1999).

Antibiotic susceptibility test

The antibiotic susceptibility test was performed on all methicillin resistant *S. aureus* screened isolates from blood culture using the Kirby-Bauer method. The antibiotics discs used for identification of antibiotic sensitivity pattern of MRSA isolates were: amoxycillin, ampicillin, gentamycin, oxacillin, penicillin-G, rifampicin, vancomycin and methicillin. The Muller Hinton agar was prepared and poured on sterile Petri plates, the blood culture isolates was taken aseptically using sterile cotton swab and swab the culture in a all over the MHA plates the plates allowed at 5 minutes for drying . Antibiotic disc were taken aseptically and placed the MHA plates. The disc was pressed gently with better contact with agar plates. The plates were incubated at 35^oc for 18 to 24 hours. After incubation antibiotic sensitivity pattern of respective organisms were identified. The results were interpreted according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 1997).

Results and discussion

Septicemia is one of the major causes of morbidity and mortality in the children and it often has a rapid and fulminant causes. The present study focused on the incidence, clinical features, bacteriological pattern and antibiotic sensitivity pattern of septicemia suspected children. A prospective study was undertaken to children age between 1-5 years admitted with clinical features and risk factors, suspected with septicemia were selected and the blood samples were collected. We identified 54 septicemia or bacteremia children out of 298 suspected children screened (18.12%). The isolated organisms from the blood sample were characterized by primary, biochemical and selective media identification methods

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(Table 1) from that identified 26 isolates *S. aureus* out of 54 positive septicemia infected children. The most predominant species was identified as *S.aureus*. Then the screening of MRSA was carried out using standard method, ten isolates of MRSA out of 26 *S. aureus* screened.

Table 1. Primary and biochemical characterization of MRSA

Isolated pathogen	Biochemical test			
	Gram staining	Oxidase test	Catalase test	Coagulase test
MRSA 1	+ve	-ve	+ve	+ve
MRSA 2	+ve	-ve	+ve	+ve
MRSA 3	+ve	-ve	+ve	+ve
MRSA 4	+ve	-ve	+ve	+ve
MRSA 5	+ve	-ve	+ve	+ve
MRSA 6	+ve	-ve	+ve	+ve
MRSA 7	+ve	-ve	+ve	+ve
MRSA 8	+ve	-ve	+ve	+ve
MRSA 9	+ve	-ve	+ve	+ve
MRSA 10	+ve	-ve	+ve	+ve

The septicemia infection is prevalent children in India, especially the age group between 1-5 years. The prevalence rate of MRSA infection in our was found to be 38.46% which is accordance with the report by Kumari *et al.*, (26.14%) in Nepal and Mehta *et al.* (1998), (32.8%) and Udaya *et al.* (1997) (20%) from India. On the contrary, some of the reports shows an alarmingly high incidence MRSA (Vidhani *et al.*, 2001; Anupurba *et al.,* 2003). An important finding of present study MRSA isolates reveals that resistant to nearly all antibiotics, sensitive to oxacillin and vancomycin (Table 2, Fig.1). This is due to the fact that MRSA is often multidrug resistant. The research findings of this resistant and sensitivity pattern are relatively similar to Mehta *et al.* (1998) and Udaya *et al.* (1997).

Table 2. Antibiotic sensitivity pattern of isolated pathogen

Isolated strain	Antimicrobial agent /symbol								
MRSA 1	Zone of inhibition (mm)	Am	A	G	Ox	P	R	Va	M
		7	11	12	13	0	10	12	0
	Response	R	R	R	S	R	R	I	R
MRSA 2	ZI	9	11	12	16	0	12	16	0
	Response	R	R	R	S	R	R	S	R
MRSA 3	ZI	7	12	10	14	0	10	15	0
	Response	R	R	R	S	R	R	S	R
MRSA 4	ZI	7	11	8	13	0	12	17	0
	Response	R	R	R	S	R	R	S	R
MRSA 5	ZI	10	10	10	15	0	12	18	0
	Response	R	R	R	S	R	R	S	R
MRSA 6	ZI	7	11	11	16	0	12	15	0
	Response	R	R	R	S	R	R	S	R
MRSA 7	ZI	7	0	9	14	0	12	14	0
	Response	R	R	R	S	R	R	I	R
MRSA 8	ZI	9	11	12	14	0	12	17	0
	Response	R	R	R	S	R	R	S	R
MRSA 9	ZI	8	12	11	15	0	12	16	0
	Response	R	R	R	S	R	R	S	R
MRSA 10	ZI	0	10	13	13	0	12	17	0
	Response	R	R	I	S	R	R	S	R

S= sensitive; R= resistant; I= intermediate; Amoxycillin (Am); Ampicillin (A); Gentamycin (G); Oxacillin (Ox); Penicillin-G (P); Rifampicin (R); Vancomycin (Va); Methicillin (M); ZI=Zone of inhibition

Conclusion

The regular monitoring of antimicrobial susceptibility pattern of MRSA and formulation of a definite antimicrobial policy may be helpful for reducing the incidence of these infections. The infected children may be isolated in separate rooms to prevent the spread of MRSA. The controlling measure need to be implemented consistently in order to reduce the burden of MRSA infection in the hospital environment. The present research suggests that oxacillin and vancomycin can be recommended for treatment of MRSA causing septicemia infection.

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Fig. 1. Antibiotic sensitivity pattern of MRSA (resistant to most antibiotics but susceptible to oxacillin and vanomycin)