INNSPUB

International Journal of Microbiology and Mycology | IJMM |
pISSN: 2309-4796
http://www.innspub.net
Vol. 4, No. 3, p. 1-7, 2016

Open Access RESEARCH PAPER

Bacteriological profile and antibiogram of blood culture isolates from patients of rural tertiary care hospital

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Key words: Antibiotics, Bacteremia, Blood culture

Publication date: October 30, 2016

Abstract

Microbial invasion of blood stream is associated with significant mortality and morbidity. Identification of bacterial isolates and antibiotic susceptibility of bacteria isolated from blood culture would guide the antibiotics treatment for patients with bacteremia. 1) To determine age - wise blood culture positivity rate in bacteremia 2) To identify age - wise common bacterial species isolates in bacteremia 3) To determine Antibiotic sensitivity pattern of the bacterial isolates. Atotal of 247 blood culture samples received from various clinical departments of rural teaching hospital from August 2013 to September 2015 were included in the study. Samples were collected in brain heart infusion broth. Identification of isolates and antimicrobial susceptibility was done as per standard microbiological methods. Out of 247 specimens bacteria sp. was isolated from 46 (18.62%) samples. Blood culture positivity was noted highest among neonates age group (38.71%). Lowest rate was observed among elders (4.55%). *Klebsiella pneumoniae*, *Coagulase negative staphylococcus (CONs)*, and *S. aureus* were common blood culture isolates. In neonates *Klebsiella pneumoniae* was the most common isolate. Out of 27 gram negative bacilli, 14 (51.85%) were extended spectrum betalactamases (ESBL) positive. High resistance was noted against amoxycillin and amoxicillin/clavulanic acid and third generation cephalosporins in all gram negative organisms except, *S. typhi*. Out of 12 *Staphylococcus sp.*, none of these were methicillin resistant. Routine antibiotic susceptibility surveillance helps in choice of antibiotics for treatment, identification of resistance and control of its spread.

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Introduction

Bacteremia is defined as presence of bacteria in blood. Microbial invasion of blood stream can have serious immediate consequences like shock, multiple organ failure, disseminated intravascular coagulation, and death.

The most common routes of entry for bacteremia are genitourinary tract (25%), respiratory tract (20%), abscesses (10%), surgical wound infections (5%), biliary tract (5%), miscellaneous sites (10%) and uncertain sites (10%).

Mortality rates associated with blood stream infections range from 20-50% (Agarwal *et al*., 2015).

Blood stream infections are very common in the in neonates and children, and has become common causes of morbidity and mortality.

In developing countries, the rate of blood stream infections in children is about 20-50% (Meremkwer *et al*., 2005; Saraswathi *et al*., 1995).

Blood stream infections require urgent and invasive management with antibiotics. Rational and appropriate use of antibiotics requires knowledge of common bacteria isolated from blood culture and their antibiotic susceptibility pattern in the region (Shreshtha *et al.,* 2014).

Resistance to various antibiotics in bacteria is major problem at present, there is wide variation seen in drug resistance, reports related to study shows variations from one region to another (Reacher *et al*., 2000; Cohen, 1997; Sobhani *et al*. 2004).

Knowledge of bacteriological profile and antibiotic sensitivity pattern would help in choice of right empiric antibiotic. Blood cultures helps in confirmation of blood stream infections, identification of causative organisms and to determine antibiotic susceptibility pattern.

Materials and methods

Sample collection and inoculation

A total 247 blood samples were received from various clinical departments from patients of bacteremia. Specimens were collected in brain heart infusion broth. The culture bottles were incubated at 37°C aerobically and periodic subcultures were done onto Mac Conkey's agar and blood agar after overnight incubation on day 1, day 2 and finally on day 7. The bacterial isolates were identified by standard biochemical tests (Collee *et al.,*1996).

Antibiotic susceptibility testing

The antibiotic susceptibility was done by modified Kirby-Bauer disc-diffusion method and zone of inhibition were measured and interpreted according to CLSI standard guidelines (NCCLS, 2000).

ESBL detection

Extended spectrum beta lactamases (ESBL) in gram negative bacterial isolates was detected using antibiotic octadiscs (Himedia) containing cefotaxime (30 µg) and cefotaxime/clavulanic acid (30/10 µg), ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg). An increase of 5 mm in the zone of inhibition in a disk containing clavulanic acid compared to the drug alone was considered as positive for ESBL producers (Thompson *et al*., 2000).

MRSA detection

Methicillin resistance in *Staphylococcus aureus* (MRSA) strains was identified using antibiotic octadiscs (Himedia) containing cefoxitin (30 µg). An inhibition zone of diameter of cefoxitin less than or equal to 21 mm indicates MRSA. *S. aureus* ATCC 25923 was used as quality control for cefoxitin susceptibility (CLSI, 2000).

Results

Total 247 blood culture specimens were received from patients of tertiary care center for culture and sensitivity. Out of 247 specimens bacteria were isolated from 46 (18.62%) blood samples and 201 (81%) blood samples were showed no growth.

Table 1. Age group-wise distribution of blood culture sample and bacterial isolates.

Age Group	Samples	Positive (Number)	Positive (Percentage)
Neonates (0-28 days)	62	24	38.71
Infants (1- 12 months)	26	5	19.23
Children (1-15 years)	55	11	20
Adults (16- 64 years)	82	5	6.09
Elders (>64 years)	22	1	4.55
Total	247	46	

Maximum numbers of samples were from Adult and Neonate age groups. Blood culture positivity was noted highest among neonates age group (38.71%) followed by infants (19.23%). Lowest rate was observed among elders (4.55%).

Table 2. Age group-wise distribution of blood culture isolates.

Bacterial isolates	Neonate	Infant	Children	Adult	Elder	Total
Klebsiella pneumoniae	12 (50)	0	4 (36.36)	0	0	16 (34.78)
CONs	2 (8.3)	1 (20)	2 (18.18)	2 (40)	0	7 (15.22)
S. aureus	3 (12.5)	0	0	1 (20)	1 (100)	5 (10.87)
Pseudomonas sp.	3 (12.5)	1 (20)	0	1 (40)	0	5 (10.87)
E. coli	1 (4.17)	2 (40)	1 (9.09)	0	0	4 (8.69)
Salmonella typhi	0	0	2 (18.18)	0	0	2 (4.35)
Enterococcus sp.	0	1 (20)	0	1 (20)	0	2 (4.35)
Acinetobacter sp.	0	0	2 (18.18)	0	0	2 (4.35)
*GNB**	2 (8.33)	0	0	0	0	2 (4.35)
Streptococcus sp.	1 (4.17)	0	0	0	0	1 (2.17)
Total	24	5	11	5	1	46 (100)

*Species were not identified by biochemical tests.

Klebsiella pneumoniae (34.78%) was the most common isolate, followed by *CONs* (15.22%), *S. aureus* (10.87%) and *Pseudomonas* sp. (10.87%). Out of 24 isolates in neonates, 12 (50%) were *Klebsiella pneumoniae*, followed by 3 (12.5%) *S. aureus* and *Pseudomonas* sp. each. Out of 5 isolates in infants, 2 (40%) were *E. coli* followed by 1 (20%) CONs, *Pseudomonas* sp. and *Enterococcus* sp. each. Out of 11 isolates in children, 4 (36.36%) were *Klebsiella pneumoniae*, followed by 2 (18.18%) CONs, *Salmonella typhi* each, and 1 (9.09%) were *E. coli* and *Acientobacter* sp. each. Out of 5 isolates in adults, 2 (40%) were CONs followed by 1 (20%), *S. aureus, Pseudomonas* sp. and *Enterococcus* sp. each. 1 isolate from elder was *S. aureus*.

Table 3. Antimicrobial sensitivity (%) pattern of GNB.

Antibiotics	*Klebsiella*	*Acinetobacter*	*E.coli*	*GNB*	*Pseudomonas*	*S. typhi*
Amoxycillin	100	100	100	100	80	0
Amoxycillin/Clavulanic acid	100	100	100	100	80	0
Ceftriaxone	100	0	75	100	60	0
Cefotaxime	100	0	75	50	20	0
Cefoperazone	100	0	75	100	20	0
Caftazidime	100	0	75	100	40	0
Cefepime	46	0	100	50	20	0
Cefixime	94	100	100	100	100	0
Piperacillin/Tazobactam	44	0	75	0	0	0
Cefoperasone/Sulbactam	75	0	50	50	0	0
Imipenem	37	0	50	0	20	0
Amikacin	75	0	0	100	0	0
Gentamicin	56	0	50	100	0	0
Tobramycin	75	0	50	100	60	0
Ciprofloxacin	39	0	25	50	20	0
Levofloxacin	10	0	0	20	0	0
Ofloxacin	50	0	0	0	20	0

Klebsiella pneumoniae and *E.coli* showed high resistance to amoxicillin (100%) and amocycilin/clavulanic acid (100%). All *Klebsiella pneumoniae* isolates showed high (100%) resistance towards third generation cephalosporins. It showed 46% resistance towards cefepime and 94% towards cefixime fourth generation cephalosporins. *Klebsiella pneumoniae* exhibited least resistance against levofloxacin (10%), imipenem (37%), ciprofloxacin (39%), piperacillin/tazobactam (44%) and cefepime (46%). High resistance was noted against amoxycillin (90.32%) and amoxicillin/ clavulanic acid (90.32%), cefotaxime (70.96%), and ceftriaxone (86.36%) in all gram negative organisms except, *S. typhi*.

ESBL Production

Out of total 27 gram negative bacilli, 14 (51.85%) were ESBL Positive. Total 14 ESBL positive gram negative bacilli of which 12 were *Klebsiella pneumoniae* and 2 were *E. coli*.

Table 4. Antimicrobial sensitivity (%) pattern of GPC.

Antiboitics	*S. aureus*	*CONs*	*Enterococcus*	*Streptococcus*
Ampicillin	80	29	100	0
Amoxy / Clavulanic acid	20	29	0	50
Cefadroxyl	20	43	100	0
Cafotaxime	40	0	100	0
Cefuroxime	20	43	0	0
Erythromycin	40	43	0	100
Azithromycin	20	59	0	100
Clindamycin	0	29	0	0
Lincomycin	20	43	0	0
Vancomycin	0	0	0	0
Teicoplanin	0	0	0	0
Linezolid	0	0	0	0
Ciprofloxacin	0	26	0	0
Ofloxacin	0	14	*	0
Tobramycin	20	0	0	0
Gatifloxacin	0	0	100	50
Cefoperazone/ Sulbactam	0	0	*	0
Roxithromycin	20	59	*	100
Cefoxitin	0	0	NA	NA

S. aureus showed high resistance (80%) towards ampicillin. All *S. aureus* were highly sensitive (100%) to vancomycin, teicoplanin, linezolid, quinolones, clindamycin and cefoperazone/ sulbactam. *Streptococcus* sp. was highly resistant (100%) to erythromycin, azithromycin and roxithromycin and 100% sensitive to ampicillin, cefodroxyl, cefuroxime, quinolones, vancomycin, teicoplanin, linezolid. *Enterococcus sp.* was 100% resistant to penicillins, cephalosporins and aminoglycosides.

Methicillin Resistance

Out of 12 *Staphylococcus sp.*, none were methicillin resistant.

Discussion

Sepsis remains one of the leading causes of death in children. Knowledge of bacterial profile and antibiotic susceptibility pattern of the isolates guide the physician for management of blood stream infections (Shrestha *et al*., 2014). The blood culture positivity rate in present study was 18.62%.

There is wide variation of blood culture positivity rate in different studies observed. Various studies have reported incidence of blood culture positivity between 10.23 to 45.9%. (Shrestha *et al*., 2014; Arpi *et al*., 1994; Colleeetal, 1996).

In the present study the rate of bacterial isolation of blood culture in neonates is 38.71%. Other studies have reported 13.3 to 50.8% neonatal blood culture positivity (Meremkwer *et al*., 2005; Shrestha *et al*., 2014; Agarwal *et al*., 2015; Onipede *et al*., 2009; Roy *et al*., 2002; Prabhu *et al*., 2010). As the age increases rate of blood culture isolation rate decreased (Prabhu *et al*., 2010).

Klebsiella pneumoniae (34.78%) was the most common isolate, followed by *CONs* 15.22%, and *S. aureus* (10.9%) in present study. In Meremikwu *et al* study, *S.aureus* (48.7%) was most common isolates, followed by coliforms (23.4%) (Meremkwer *et al*., 2005) Knowledge of common bacteria isolated from blood culture, and their antimicrobial susceptibility in a given area helps to inform the choice of antibiotics.

In neonates, the common isolates of blood culture in present study were *K. pneumoniae*11 (50%) and S*. aureus* (12.5%). The Avinikaagarwal *et al* study reported *K. pneumoniae* as most common isolate (Agarwal *et al*., 2015). *Staphylococcus aureus* was the most common isolate in neonates in other studies. (Meremkwer *et al*., 2005; Onipede *et al*. 2009; Prabhu *et al*. 2010) Knowledge of bacteriological profile in given area is required as clinicians would not wait for culture and sensitivity results for starting antibiotic therapy as neonatal septicemia is associated with high rate of mortality and morbidity and right choice of antibiotic at right time is very important.

All *Klebsiella pneumoniae* isolates exhibited highest (100%) resistance against amoxycillin, amoxycillin/ clavulanic acid and lowest resistance against levofloxacin (10%), imipenem (37%), ciprofloxacin (39%), piperacillin/tazobactam (44%) and cefepime (46%). In present study *Klebsiella pneumoniae* was the most common isolate in neonate as well as in overall blood culture isolates, knowledge of antibiotic susceptibility pattern would help in treating emergency condition like neonatal septicemia.

Chloramphenicol, cephalosporins, and fluroquinolones were very effective against *S. typhi* (100%). In present study gram negative bacilli except *S. typhi* showed highest resistance against amoxycillin and amoxycillin/clavulanic acid and lowest resistance showed against imipenem, piperacillin/tazobactam, and levofloxacin. In present study, Out of 27 gram negative bacilli, 14 (51.85%) were ESBL positive. Out of 16 *Klebsiella pneumoniae*, 12 were ESBL positive. In Prabhu *et al* study, prevalence of ESBL producer gram negative bacteria was 32% (Prabhu *et al*. 2010). Continuous screening and surveillance for detection of ESBL producers gram negative bacteria in hospital is necessary.

In present study, *S. aureus* showed high resistance towards ampicillin, *Streptococcus* sp*.* was highly resistant to erythromycin, azithromycin and roxithromycin, and Enterococcus sp. was highly resistant to penicillins, cephalosporins and aminoglycosides.

All gram positive cocci isolates were susceptible to vancomycin, teicoplanin, linezolid and cefoperazone/ sulbactam. Out of 12 *Staphylococcus* sp*.,* none were methicillin resistant. The incidence of MRSA was between 29 - 57% in other studies (Shrestha *et al*., 2014; Agarwal *et al*., 2015; Prabhu *et al*., 2010).

Antibiogram of isolates varies from region to region. Knowledge of antibiotic sensitivity pattern would help in choice of right empiric antibiotic as it is an emergency condition. Increase resistance in these common pathogens to common antibiotics calls for increased efforts to ensure more rational use of these drugs. In the management of sepsis in pediatric age group, empirical antibiotic therapy should be determined by the prevalent spectrum of etiological agents and their antibiotic sensitivity pattern. Present study suggest the need for periodic survey of causative agents and their resistance surveillance reports which can would guide appropriate choice of empiric therapy.

Conclusion

Routine antibiotic susceptibility surveillance of blood culture isolates helps in choice of antibiotics for treatment, identification of resistance and control of its spread.

Limitation:

The data in this study are based on a single blood culture and no data were collected regarding prior antibiotic usage that may have effect on blood culture positivity rate.

key message

Knowledge of bacteriological profile and antibiotic sensitivity pattern would help in choice of right empiric antibiotic as bacteremia particularly in neonates is emergency condition.

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