



BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ESKAPE PATHOGENS IN BLOOD CULTURE ISOLATED FROM A TERTIARY CARE CENTRE.

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ABSTRACT

Bacteraemia and septicaemia are a major health problem, which leads to high morbidity and mortality of patients. Timely diagnosis and appropriate medication will be the best way to save the lives of affected ones. The aim of the present study was to determine the bacterial profile of suspected bacteraemia cases and antibiotic susceptibility pattern of ESKAPE pathogens. This is a retrospective study of 3684 blood samples collected from clinically suspected cases of bacteraemia, over a period of two years (April 2015 to March 2017). The isolates were identified by conventional biochemical tests and antimicrobial susceptibility testing by CLSI guidelines. Positive cultures were obtained in 240 (6.5%) cases of which ESKAPE organisms accounted for 66% of cases with *Pseudomonasaeruginosa* 34% (81) predominance, non ESKAPE organisms accounted were 34% of cases with *E. coli* 15% (36) predominance and one fungal isolates. Antibiotic resistant pattern among ESKAPE pathogens were, among *Enterococcus faecium* only one was resistant to vancomycin, among *Staphylococcus aureus* Methicillin esistant *taphylococcus aureus* resistant was observed. *Klebsiella* spp resistant to piperacillin tazobactam and cefaperazone sulbactam, *Acinetobacter baumannii* and *P. aeruginosa* ciprofloxacin showed highest activity followed by cefoperazone and sulbactam. *Enterobacter* spp showed no resistance. This study highlights the health crisis & emergence of ESKAPE pathogens as predominant cause of bacteraemia. It is necessary that the last remaining antimicrobial agents be protected via intellectual choice and ameliorated infection control. Prescription of the most appropriate antibiotics at the right dose and period will consistently improve the sick and decrease the appearance of antibiotic resistance.

KEYWORDS: *Bacteraemia, Septicaemia, Febrile patients, Antimicrobial susceptibility pattern, ESKAPE pathogens.*



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INTRODUCTION

Blood stream infections are an important cause of mortality and morbidity among common health care associated infections.¹ Blood stream infection ranges from self-limiting infections to life threatening sepsis that needs rapid treatment.² Bacteraemia is the presence of viable bacteria in the blood stream. Where else sepsis/blood poisoning /toxaemia/septicaemia, is a clinical syndrome characterized by fever, chills, malaise, tachycardia, hyperventilation, and toxicity or prostration, where there is rapid multiplication of bacteria. Bacteraemia may be transient, intermittent or continuous. Transient bacteraemia means organisms from the normal flora, are introduced into the blood by minimal trauma to membranes (eg, brushing of teeth, straining during bowel movements or medical procedures.)³ Intermittent which means bacteria from an infected site are periodically released into the blood from extravascular abscesses, spreading cellulitis, or infections of body cavity, such as empyema, peritonitis, or septic arthritis.⁴ Continuous bacteraemia usually occurs when the infection is intravascular, such as infective endothelium or infected hardware (AV fistula, indwelling cannulas).⁴ Bacteraemia may result from an infection in an organ or tissue (secondary bacteraemia), if the primary site is not evident, then it's called primary bacteraemia.⁴ Several mechanisms play a role in eliminating the bacteria from the bloodstream. In healthy and immunocompetent hosts bacteria are eliminated from the blood stream within 30 to 45 minutes, where else in immunocompromised hosts it takes longer time for elimination.⁴ Antimicrobial resistance is one of the important health concerns. The occurrence of multidrug resistant pathogens in hospital and community has been on rise in the last decade along with nosocomial infections. High frequency of multi drug resistant pathogens that have been grouped under the acronym ESKAPE comprising: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. The ESKAPE pathogen causes nosocomial infections and capable of 'escaping' the biocidal action of antibiotics.⁵ The timely detection of bacteraemia, followed by expeditious identification of pathogens and determination of susceptibility to antimicrobial agents can have great diagnostic and prognostic importance. Prompt initiation of appropriate antimicrobial therapy is demonstrably important for preventing morbidity and mortality. The purpose of this study was to know the bacterial profile and antimicrobial susceptibility pattern of culture positive ESKAPE pathogens isolated in blood, from patients admitted at Saveetha Medical College and Hospital, Thandalam, Kancheepuram Dt, Tamilnadu.

MATERIALS & METHODS

Study design and data collection

This study was carried out in different ICUs, medicine ward, surgery ward, ortho ward, OBG ward tertiary care medical college & hospital from April 2015 to March 2017. During this period, 3684 patients were suspected of having bacteraemia and the required blood samples were drawn prior to the administration of antibiotics.

Blood collection and bacterial identification

Five millilitres of venous blood was collected from all suspected adult patients by nursing personnel, male orderlies, or physicians, under strict aseptic precautions. It was inoculated into 50 ml of conventional blood culture bottles⁶ containing Brain Heart infusion (BHI) broth with 0.025% of sodium polyanethol sulphate as anticoagulant (Hi – Media, India). Blood culture samples were incubated at 37^o C upto 7 days, and subculture were performed on Blood agar, Chocolate agar, MacConkey agar on the 2nd, 4th and 6th day of incubation as per the standard protocol.⁶ Isolates from blood culture samples (240/3684) were identified based upon gram staining characteristics, colony morphology, motility, oxidase test, catalase test and a panel of biochemical test.⁷

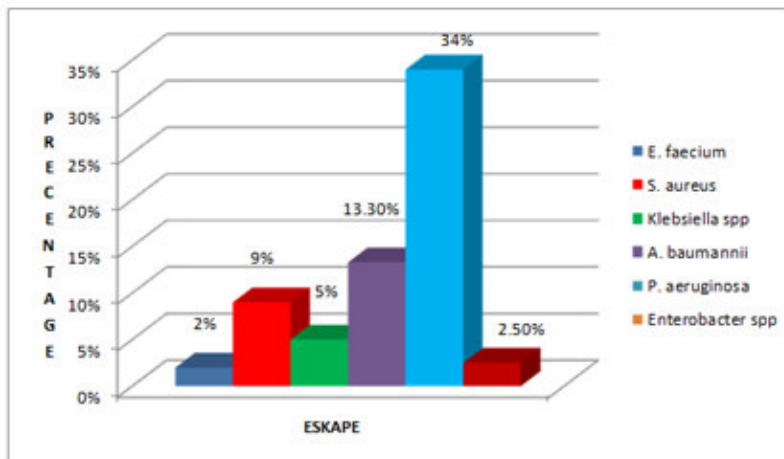
Antimicrobial susceptibility test and Quality control

Following identification of the bacterial isolates, antibiotic susceptibility testing was performed on Mueller-Hinton agar plates by Kirby-Bauer disk diffusion method as per Clinical Laboratory Standard Institute 2012 guidelines.⁸ The antibiotics discs and their concentrations for gram positive bacteria included ; *Erythromycin* (15µg), *Clindamycin* (2µg), *Ampicillin* (10µg), *Penicillin* (10U), *Cefazolin* (30 µg), *Cefoxitin* (30µg), *Ceftriaxone* (5µg), *Ofloxacin* (5 µg), *Ciprofloxacin* (5µg), *Gentamicin* (10µg), *High level Gentamicin* (120 µg) *Cotrimoxazole* (30µg), *Tetracycline* (30µg), *Vancomycin* (30µg) and *Linezolid* (30µg) . For Gram negatives *Gentamicin* (10 µg), *Amikacin* (30 µg), *Ampicillin* (10µg), *Cefazolin* (30 µg) , *Cefotaxime* (30 µg), *Cefepime* (30 µg), *Ceftazidime* (30 µg), *Cefaperazone – Sulbactam* (75 µg), *Piperacillin – Tazobactam* (100/10 µg), *Imipenem* (10 µg), *Meropenem* (10 µg), *Ciprofloxacin* (5µg), *Ofloxacin* (5 µg), *Cotrimoxazole* (1.25/23.75 µg), *Colistin* (10 µg), *Polymyxin B* (300 U), discs were used. These were procured from Hi- Media. The reference strains used as control for disc diffusion testing were *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 and *E. faecalis* ATCC 29212

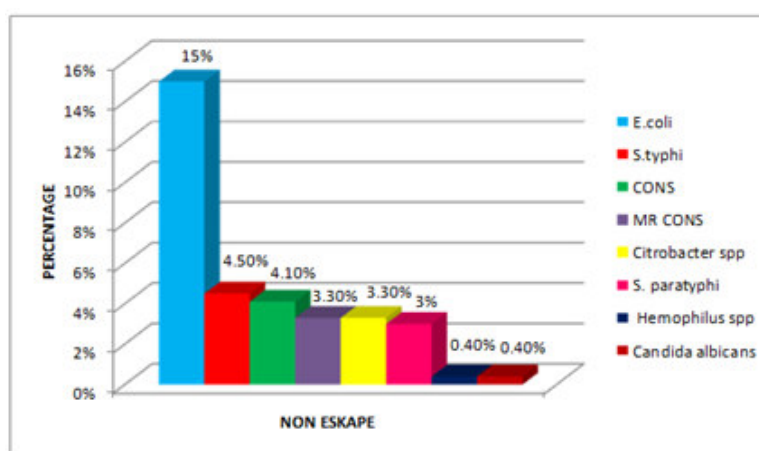
RESULTS

Characteristic of study population

During the study period 3684 blood samples from clinically suspected cases of bacteraemia, 240 were culture positive. Of this, 160 (67%) were males and 80 (33%) were females resulting in a ratio of 2:1. The predominant age group being affected were 35 – 45 years. Among the 240 positive cultures, 40 were from ICU and 200 were from wards. Of the total 240 positive blood culture, ESKAPE and non ESKAPE pathogens were 158 (66%) and 82 (34%) respectively. Among the ESKAPE pathogens, *E. faecium* 2%(4), *S. aureus* 9 %(22), *Klebsiella* spp 5 % (13), *A. baumannii* 13.3 % (32), *P. aeruginosa* 34% (81) and *Enterobacter* spp 2.5% (6). (Graph 1). Among non ESKAPE pathogens, *Escherichia coli* 15% (36), Coagulase Negative *Staphylococcus* (CONS) 4.1% (10), Methicillin resistant CONS 3.3% (8), *Salmonellatyphi* 4.5% (11), *Citrobacter* spp 3.3 % (8), *Salmonella paratyphi* 3% (7) , *Hemophilus* spp 0.4% (1) , *Candida albicans* 0.4% (1). (Graph 2)



Graph 1
Distribution of ESKAPE pathogens isolated in blood culture during a two year study period



Graph 2
Distribution of non ESKAPE pathogens isolated in blood culture during a two year study period

Table 1
Antimicrobial resistance pattern of ESKAPE pathogens isolated from blood culture.

Antimicrobial drugs	Resistant bacterial isolates (%)					
	<i>E. faecium</i> N = 4	<i>S. aureus</i> N = 22	<i>Klebsiella</i> spp N = 13	<i>A. baumannii</i> N = 32	<i>P. aeruginosa</i> N = 81	<i>Enterobacter</i> spp N = 6
Penicillin	0%	81.8%				
Ampicillin	50%		100%			0%
Vancomycin	25%					
Gentamicin	0%	54.5%	46.1%	18.7%	38.2%	50%
Linezolid	0%					
High level Gentamicin	25%					
Erythromycin		54.5%				
Cefoxitin		22.7%				
Tetracyclin		0%				
Cotrimoxazole		0%	61.5%	6.2%		16.6%
Amikacin			46.1%	22%	30.8%	16.6%
Cefazoline			0%			0%
Cefotaxime			0%	6.2%		0%
Cefipime			0%	0%	12.3%	0%
Cefaperazone Sulbactam			61.5%	0%	19.7%	0%
Piperacillin			76.9%	31.2%	5%	0%
Imipenem			23%	34.3%	6.1%	0%
Meropenem			23%	37.5%	0%	
Ciprofloxacin			61.5%	0%	39.3%	16.6%
Ceftazidime					44.4%	0%

DISCUSSION

Health crisis of ESKAPE pathogens seems overwhelming. The present study broadly illustrates the blood stream infection specially ESKAPE pathogens and its antimicrobial susceptibility pattern. The observed blood culture positivity rate in this study was 6.5% which is low compared to other studies where the reported isolation of organisms was 9.9 %⁹, 20.5 %⁵, 9.2%¹⁰, 27.9%¹¹. The variation may be due to the different methodology used, place of study, because of the regional variation known to occur. In this present study, 79% of infections were caused by gram negative bacilli and 21% by gram positive cocci, which is almost similar with other studies where the results of isolation of gram negative bacilli and gram positive cocci were (81% and 18%)⁹, (67.5% and 32.5%)⁵, (51.8% and 46.4%)¹¹, Nepal (89.19% and 10.81%) (David *et al.* 2004)¹⁵ and Saudi Arabia (62.2% and 33.8%) (Elbasher *et al.* 1998)¹⁶, respectively. Though there are studies which reported gram positive cocci is more common than gram negative bacilli where the isolation was (58.3% and 40.2%)¹⁰, (57.8% and 42.2%)¹², in USA (65% and 25%)¹⁷, Iran (72% and 28%)¹⁸ and UK (66.2% and 31.3%)¹⁹ however in our present study gram negative bacilli have been encountered more from blood cultures than gram positive cocci. In our study, the incidence of ESKAPE pathogens is 158/240 (65.8%) and non ESKAPE pathogens were 82/240 (34.1%). In our present study *E. faecium* was isolated in 2% (n=4) of cases which is similar to other studies where the reported isolation of the organism was (2.3%)⁹, (3.7%)⁵, (3.8%)¹⁰ and (1%)¹¹. *S. aureus* was isolated in 9% (n=22) of cases which is similar to (8.3%)⁵, (11.8%)¹² and (10.3%)¹³ but contrary to other studies where the reported isolation of the organism was, (14%)⁹, (38.6%)¹⁰ and (23.2%)¹¹. *Klebsiella pneumoniae* was isolated in 5% (n=13) of cases which is relatively less when compared with others studies where reported isolation of the organism was (15%)⁹, (7.3%)⁵, (9.8%)¹⁰, (16%)¹¹ and (48.4%)¹⁴. *A. baumannii* was isolated in 13.3% (n=32) of cases which is similar to (12.6%)⁵, but contrary to other studies where reported isolation of the organism was, (1.5%)¹⁰, (1.9%)¹², (0.8%)¹³ and (3.3%)¹⁴. *P. aeruginosa*, was isolated in 34% (n=81) of cases which is more compared with other studies where reported isolation of the organism was (19.7%)⁹, (16%)⁵, (5.3%)¹⁰, (3.1%)¹², (0.2%)¹³ and (4.6%)¹⁴. *Enterobacter* spp was isolated in 2.5% (n=6) of cases which is similar to other studies where reported isolation of the organism was (3.5%)¹¹, (1.9%)¹² and (4.6%)¹⁴. Among the *E. faecium* (4), only 1 were vancomycin resistant (Table 1), which is similar to 16.6 %⁵ and in

accordance with Kalpesh *et al.*¹⁰ Among the *S. aureus* (22), 5 (22%) were resistance to *Cefoxitin* (Table 1) which indicates MRSA which is in accordance with Kamga *et al.*²⁰ where reported MRSA¹¹ was 18% but less when compared with other studies where it was reported 75.6%⁵ and 70.6%¹⁰. The increasing resistance in our study could be due to widespread usage of drugs in the treatment protocol of suspected bacteraemia. The current resistance pattern emphasizes the importance of strict antibiotic policy to prevent emergence and spread of antibiotic resistance. Among the *Klebsiella pneumoniae* (13), 100% resistant to ampicillin (Table 1) which is in accordance with Atul *et al.*⁵, 76.9% resistant to piperacillin tazobactam and 61.5% resistant to cefoperazone sulbactam (Table 1) which is more when compared with Atul *et al.*⁵. So 100% ESBL was observed in our study. Among the Non Fermenters *A. baumannii* and *P. aeruginosa*, ciprofloxacin showed highest activity (100%) followed by cefoperazone and sulbactam (Table 1) showed 100% activity similar to Manjula *et al.*⁹ and Atul *et al.*⁵ studies. Among the *Enterobacter* spp (8) were sensitive to all drugs (Table 1), however it's contradicting to Gupta *et al.*¹⁴ study which showed resistant strain. However it is apparent that surveillance program like using standardize laboratory methods, establish systems to notify infection control staff when resistance pattern is detected, develop and implement laboratory protocol for storing isolates of resistant organisms for molecular typing when needed to confirm and to develop and monitor specific antimicrobial susceptibility reports for early identification of changes in the spectrum of microbial pathogens, risk factors causing them.

CONCLUSION

The present study data provided needed information on the prevalence of antimicrobial resistance amongst the ESKAPE pathogens causing bacteraemia and septicaemia. The increase in resistance in blood isolates emphasises the importance of early hospital infection control, rational prescribing policies, and the need for newer drugs and vaccines. The results were helpful in proving useful guidelines for choosing an effective and appropriate antibiotics covering both ESKAPE and non ESKAPE pathogens causing bacteraemia and septicaemia, and for choosing therapy against hospital resistant strains.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. *J Clin Microbiol* 2003 Aug 1;41(8):3655-60.
2. Zembower TR. Epidemiology of infections in cancer patients. In *Infectious Complications in Cancer Patients*, Springer International Publishing. 2014 p.43-89.
3. LeFrock JL, Ellis CA, Turchik JB, Weinstein L. Transient bacteremia associated with sigmoidoscopy. *Ann Intern Med.* 1973 May 1;78(5):826.
4. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. *Diagnostic microbiology. The nonfermentative gram-negative bacilli.* Philadelphia: Lippincott-Raven Publishers. 1997. p.253-320.
5. Garg A, Anupurba S, Garg J, Goyal RK, Sen MR. Bacteriological profile and antimicrobial resistance

- of blood culture isolates from a university hospital. *Journal of Indian Academy of Clinical Medicine*. 2007 Apr;8(2):139-43.
6. Collee JG, Miles RS, Watt B. Tests for identification of bacteria. *Mackie and McCartney practical medical microbiology*. 1996;14:131-49.
 7. Winn Washington C, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, Woods GL. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. Lippincott, Williams & Wilkins; 2006.
 8. Edition AS. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute. 2012;32(1):76.
 9. Mehta M, Dutta P, Gupta V. Antimicrobial susceptibility pattern of blood isolates from a teaching hospital in North India. *Jpn J Infect Dis*. 2005 Jun 1;58(3):174.
 10. Gohel K, Jojera A, Soni S, Gang S, Sabnis R, Desai M. Bacteriological profile and drug resistance patterns of blood culture isolates in a tertiary care nephrourology teaching institute. *BioMed research international*. 2014 Apr 7;2014.
 11. Negussie A, Mulugeta G, Bedru A, Ali I, Shimeles D, Lema T, Aseffa A. Bacteriological profile and antimicrobial susceptibility pattern of blood culture isolates among septicemia suspected children in selected hospitals addis ababa, ethiopia. *Int J Biol Med Res*2015 Nov;6(1):4709.
 12. Prakash KP, Arora V, Geethanjali PP. Bloodstream bacterial pathogens and their antibiotic resistance pattern in Dhahira Region, Oman. *Oman Med J*. 2011 Jul;26(4):240.
 13. Wasihun AG, Wlekidan LN, Gebremariam SA, Dejene TA, Welderufael AL, Haile TD, Muthupandian S. Bacteriological profile and antimicrobial susceptibility patterns of blood culture isolates among febrile patients in Mekelle Hospital, Northern Ethiopia. *Springer Plus*. 2015 Dec 1;4(1):314.
 14. Gupta N, Choudhary U, Garg N, Arora DR. *Enterobacter bacteremia*. *J Assoc Physicians India*2003 Jul;51:669-72.
 15. Murdoch DR, Woods CW, Zimmerman MD, Dull PM, Belbase RH, Keenan AJ, Scott RM, Basnyat B, Archibald LK, Reller LB. The etiology of febrile illness in adults presenting to Patan hospital in Kathmandu, Nepal. *Am J Trop Med Hyg*2004 Jun 1;70(6):670-5.
 16. Elbashier AM, Malik AG, Knot AP. Blood stream infections: micro-organisms, risk factors and mortality rate in Qatif Central Hospital. *Ann Saudi Med* 1998;18(2):176.
 17. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004 Aug 1;39(3):309-17
 18. Mamishi S, Pourakbari B, Ashtiani MH, Hashemi FB. Frequency of isolation and antimicrobial susceptibility of bacteria isolated from bloodstream infections at Children's Medical Center, Tehran, Iran, 1996–2000. *Int J Antimicrob Agents*. 2005 Nov 30;26(5):373-9.
 19. Gray JW. A 7-year study of bloodstream infections in an English children's hospital. *Eur J Pediatr*. 2004 Sep 1;163(9):530-5.
 20. Kamga HL, Njunda AL, Nde PE, Assob JC, Nsagha DS, Weledji P. Prevalence of septicaemia and antibiotic sensitivity pattern of bacterial isolates at the University Teaching Hospital, Yaoundé, Cameroon. *African Journal of Clinical and Experimental Microbiology*. 2011;12(1).

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