



RESEARCH PAPER

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Epidemiological studies of antibiotic resistance in pathogens isolated from blood cultures of people from Cuddalore District

C. Uma Maheswari¹, V. Arul Balaji², J. Vigneshwari³, G. Vinitha², M. Shenbagam^{*1}

¹Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar, Tamilnadu, India

²Agenesinfo Omics Pvt. Ltd., Hyderabad, India

³Project Fellow, Rusa 2.0, Department of Microbiology, Annamalai University, Annamalai Nagar, Tamilnadu, India

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Abstract

Infections due to multidrug-resistant (MDR) microorganisms have triggered antimicrobial resistance among the antibiotics pattern. Antimicrobial resistance (AMR) in pathogens isolated from blood cultures is currently one of the most concerning issues in medicine. This study was to investigate the AMR distribution of the blood-borne pathogens isolated over two years in the Cuddalore region. A surveying laboratory-confirmed bloodstream infection (BSIs) was done and data from various major diagnostic laboratories were used. During the study period, 390 positive BSIs were collected. The prevalence of gram-positive bacteria (51%) and gram-negative bacteria (49%). The most common pathogens were gram-positive isolates *Staphylococcus haemolyticus* (38%) and *Staphylococcus hom. hominis* (31%) and gram-negative isolates *Enterobacter cloacae* complex (13%), *Acinetobacter haemolyticus* and *Klebsiella pneumonia* (18%). The gram-positive *Staphylococcus haemolyticus* and *Staphylococcus hom. hominis* showed the highest resistance and sensitivity antibiotics pattern. Tigecycline was a highly sensitive antibiotic against all gram-positive isolates. *Citro. Koseri* showed sensitivity among the gram-negative antibiotic pattern. High resistance rates to both frontline antibiotics were observed for all gram-positive organisms isolated from blood cultures.

* Corresponding Author: Dr. M. Shenbagam ✉ shenbu15@gmail.com

Introduction

Antimicrobial resistance has faced different alterations, in a family of antibiotics, sensitivity, and resistance patterns to various antibiotics due to different causes such as over-the-counter use, and the non-prescription utility of many antibiotics (A. Saeidinia *et al.*, 2016, F. Saeidinia *et al.*, 2014). However; multidrug resistance infections of the blood lead to more death and complications in hospitalized patients (M. Alagesan *et al.*, 2015). A lack of national antibiotic resistance monitoring in developing countries will lead to unsuitable use in people and the healthcare system (F. Prestinaci *et al.*, 2015).

The long-term hospitalization of patient infection caused by multidrug resistance increases mortality and the risk of other infections (A. Revelas 2012). Antibiotic resistance is a general concern worldwide, and globally increasing antibiotic resistance is different by region and according to geographic and epidemiological features (X. Zhang *et al.*, 2015, A. Saeidinia *et al.*, 2016). Indian population is known to be the highest consumer of antibiotics in the world (R. Laxminarayan and R. Chaudhury 2017).

The Government of India has developed a National Action Plan on Antimicrobial Resistance (NAP-AMR) 2017–2021(NAP-AMR). The Indian Council of Medical Research (ICMR) has established an Antimicrobial Resistance Surveillance & Research Network (AMRSN) across selected hospitals in India, focusing on drug resistance among six pathogens (K. Walia *et al.*, 2019). Therefore, this investigation analyzed the pattern of antibiotic resistance for different organisms in a beneficial model for clinicians.

Materials and methods

Study Design

A reflective electronic record review of laboratory-confirmed bloodstream infections (BSIs) described the AMR among key bacterial microorganisms.

The collected data were systematized by their gram-positive and gram-negative microorganisms and the antibiotic susceptibility profile.

Sample Preparation

The blood samples for culture were obtained from patients in BacT/Alert FAN Plus media bottles and incubated in a BacT/Alert system (bioMérieux, Florence, Italy). Positive BacT/Alert blood samples were processed for Gram staining and subcultured on MacConkey and Blood agar media. After the first culture was negative, the second sampling was obtained in the following 72 hours and the final culture sampling was done after 10 days of obtaining negative results from the second culture. The positive cultures resulted in colonies. All positive blood cultures in the laboratories were assessed and assayed for antibiotic susceptibilities. The antibiotic susceptibilities of clinical isolates were determined using the automated Vitek®2 system (bioMérieux, Florence, Italy) as per manufacturer guidelines.

Statistical Analysis

GraphPad 7 was used for data analysis in all steps. Quantitative data were indicated as mean qualitative variables as frequency and percentages.

Results

During the study period, 390 positive blood cultures were obtained. The majority of positive isolates were male and others were female. The frequency of bacteria isolated from the blood cultures of patients is shown in Table 1. The prevalence of gram-positive bacteria (51%) and Gram-negative bacteria (49%).

Table 1. Distribution of bacteria in blood culture cases.

Bacteria isolated from the blood cultures	Frequency	Percent%
Gram-negative bacteria		
<i>Acinetobacter baumannii</i>	15	4
<i>Acinetobacter haemolyticus</i>	32	8
<i>Ent.cloacae complex</i>	50	13
<i>Ewingella americana</i>	16	4
<i>Klebsiella oxytoca</i>	16	4
<i>Klebsiella pneumoniae</i>	32	8
<i>Citrobacter koseri</i>	16	4
<i>Sphingomonas paucimobilis</i>	15	4
Gram-positive bacteria		
<i>Enterococcus faecium</i>	24	6
<i>Enterococcus faecalis</i>	20	5
<i>Staphylococcus aureus</i>	25	7
<i>Staphylococcus haemolyticus</i>	72	19
<i>Staphylococcus hom.hominis</i>	44	11
<i>Staphylococcus warneri</i>	13	3
Total	390	100

Gram-Positive Amr Pattern

The dominant prevalence of Gram-positive bacteria was 51%. Among all isolates, gram-positive bacteria species most frequently found were: *Enterococcus faecium* (6%), *Enterococcus faecalis* (5%), *Staphylococcus aureus* (7%), *Staphylococcus haemolyticus* (19%), *Staphylococcus hominis* (11%), and *Staphylococcus warneri* (3%) (Table 1). The antibiotic pattern of gram-positive bacteria showed in Table 2.

Table 2. Antibiotic pattern of gram-positive bacteria from Blood isolates.

Bacteria isolated from the blood cultures	Antibiotic Pattern		
	Sensitive, n%	Intermediate, n%	Resistance, n%
Gram-positive bacteria			
<i>Enterococcus faecium</i>	11 (24)	0 (0)	13 (29)
<i>Enterococcus faecalis</i>	12 (27)	0 (0)	8 (18)
<i>Staphylococcus aureus</i>	12 (27)	0 (0)	13 (29)
<i>Staphylococcus haemolyticus</i>	27 (60)	0 (0)	45 (100)
<i>Staphylococcus hominis</i>	14 (31)	0 (0)	40 (89)
<i>Staphylococcus warneri</i>	0 (0)	0 (0)	13 (29)

The gram-positive isolates *Staphylococcus haemolyticus* and *Staphylococcus hom. hominis* showed the highest resistance antibiotics pattern. However; *Staphylococcus haemolyticus* and *Staphylococcus hom. hominis* reposed the highest sensitives antibiogram.

The *Enterococcus faecium*, *Enterococcus faecalis*, and *Staphylococcus aureus* significantly showed a sensitivity of 27%. The *Staphylococcus warneri* was only the bacteria completely resistant against all the gram-positive selected antibiotics.

The antibiotic discs in antibiograms and their patterns showed in Table 3. Among the gram-positive isolates, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, and *Staphylococcus warneri* were resistant to Gentamicin, Levofloxacin, Ciprofloxacin, Benzyl Penicillin, and Clindamycin respectively.

Tigecycline, Ciprofloxacin and Trimethoprim/Sulphamethoxazole showed the highest significant sensitivity against *Entero.faecium*, *Entero.faecalis*, and *Staph. aureus*.

Table 3. Antibiotic discs and their patterns.

Gram Positive	Sensitive	Intermediate	Resistance
Antibiotic			
Benzyl Penicillin	2	0	5
Ciprofloxacin	11	1	11
Clindamycin	3	0	5
Gentamicin	9	0	14
Gentamicin high level	1	0	1
Levofloxacin	9	2	13
Linezolid	5	0	3
Nitrofurantoin	8	0	2
Oxacillin	2	0	4
Rifampicin	3	0	2
Teicoplanin	5	0	1
Tetracycline	4	0	2
Ticarcillin/Clavulanic Acid	1	1	1
Tigecycline	13	0	1
Trimethoprim	7	1	2
Trimethoprim/Sulphamethoxazole	10	0	4
Vancomycin	5	0	1

Gram-Negative Amr Pattern

The prevalence of gram-negative bacteria was 49%. The frequency of gram-negative isolated from the blood cultures of patients showed in Table 1. Gram-negative bacteria species most frequently found were: *Acinetobacter baumannii* (4%), *Acinetobacter haemolyticus* (8%), *Ent. cloacae* complex (13%), *Ewingella americana* (4%), *Klebsiella oxytoca* (4%), *Klebsiella pneumonia* (8%), *Citrobacter koseri* (4%), and *Sphingomonas paucimobilis* (4%). The *Ent. cloacae* complex (51%), *Ewingella americana* (29%), *Acinetobacter haemolyticus*, and *Klebsiella pneumonia* (20%). The antibiotic pattern of gram-negative isolates showed in Table 4.

Table 4. Antibiotic pattern of gram-negative bacteria from Blood isolates.

Bacteria isolated from the blood cultures	Antibiotic Pattern		
	Sensitive, n%	Intermediate, n%	Resistance, n%
Gram-negative bacteria			
<i>Acinetobacter baumannii</i>	14 (31)	0 (0)	1 (2)
<i>Acinetobacter haemolyticus</i>	20 (44)	3 (7)	9 (20)
<i>Ent.cloacae complex</i>	25 (56)	2 (4)	23 (51)
<i>Ewingella americana</i>	3 (7)	0 (0)	13 (29)
<i>Klebsiella oxytoca</i>	16 (36)	0 (0)	0 (0)
<i>Klebsiella pneumoniae</i>	18 (40)	5 (11)	9 (20)
<i>Citrobacter koseri</i>	16 (36)	0 (0)	0 (0)
<i>Sphingomonas paucimobilis</i>	15 (33)	0 (0)	0 (0)

Amikacin, Ampicillin/Sulbactam, Cefepime, Colistin, Imipenem, and Doripenem were significantly sensitive among the gram-negative isolates. Ampicillin/Clavulanic acid, Cefuroxime, Meropenem, Aztreonam, and Nitrofurantoin were resistant to

Acinetobacter baumannii, *Acinetobacter haemolyticus*, and *Citrobacter koseri* respectively. The *Ent. cloacae* showed more resistance among the gram-negative isolates. The gram-negative antibiotics disc pattern showed in Table 5.

Table 5. Antibiotic discs and their patterns

Gram Negative			
Antibiotic	Sensitive	Intermediate	Resistance
Ampicillin/Clavulanic acid	6	0	2
Ampicillin/Sulbactam	8	0	3
Amikacin	10	0	2
Aztreonam	6	0	6
Cefalexin	2	0	6
Cefazolin	2	0	6
Cefepime	8	0	5
Cefixime	5	0	6
Cefotaxime	4	0	6
Cefpodoxime	8	0	6
Ceftazidime	7	0	6
Ceftriaxone	1	0	6
Cefuroxime	6	0	7
Colistin	8	3	1
Daptomycin	6	0	2
Doripenem	8	0	4
Erythromycin	2	0	7
Imipenem	9	0	2
Meropenem	7	1	3
Minocycline	7	0	2
Ofloxacin	1	0	2

Discussion

The estimation of antibiotic resistance patterns is very important in developing countries like India. This study was to investigate the distribution and frequency of blood pathogens and their antimicrobial resistance patterns. The current study revealed the patterns of resistance and sensitivity of blood infection in the area of the Cuddalore region. WHO indicated the highest carbapenem resistance worldwide in *Acinetobacter baumannii* (91%) and *Pseudomonas aeruginosa* (82%) (WHO 2019). Another study suggested that carbapenem resistance prevalence in *Klebsiella pneumoniae* (40–50%) but a significantly lower level in *E. coli* (15–25%) in the general population. The high rate of antimicrobial resistance in bloodstream infections among infants was *Staphylococcus aureus*, and 42.5% were methicillin-resistant among children in India.

In India currently, resistance rates of older antibiotics ampicillin and trimethoprim-sulfamethoxazole were decreased and increased susceptibility (G. Sumanth *et al.*, 2014). These changes are due to the replacement of these drugs as empiric treatment (V.V. Mogasale *et al.*, 2021).

The current study's high prevalence of gram-positive isolates was *Staphylococcus haemolyticus*, *Staphylococcus hom. hominis*, and *Staphylococcus warneri*. Those isolated AMR profiles showed resistance against Gentamicin, Levofloxacin, Ciprofloxacin, Benzyl Penicillin, and Clindamycin respectively. Cefpodoxime shows high resistance against *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, and *Staphylococcus hom.hominis*. Tigecycline was only a highly sensitive antibiotic against all gram-positive isolates. *Sphingomonas paucimobilis* highly sensitive among all antibiotics. The *Staphylococcus warneri* was only the bacteria completely resistant against all the gram-positive selected antibiotics.

Tigecycline, Ciprofloxacin, and Trimethoprim/Sulphamethoxazole showed the highest significant sensitivity against *Enterococcus faecium*, *Enterococcus faecalis*, and *Staphylococcus aureus*. The frequency of gram-negative isolates was *Ent. cloacae* complex (51%), *Ewingella americana* (29%), *Acinetobacter haemolyticus*, and *Klebsiella pneumonia* (20%). Colistin was highly responded against *Klebsiella pneumonia*. *Citrobacter koseri* was high sensitivity among the gram-negative antibiotic pattern. *Ewingella americana* was a high-resistance isolate in gram-negative bacteria. High resistance rates to both frontline antibiotics and those of last resort were observed for all gram-positive organisms isolated from blood cultures.

Conclusion

Staphylococcus spp. *Enterobacter cloacae* and *Ewingella americana* are common pathogens associated with BSI in the Cuddalore region and they are resistant to several antibiotics. Most of the gram-positive and negative isolates are resistant to common antibiotics. Continuous AMR surveillance will warrant to reduce of the resistant strains.

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Competing interests

Authors have declared that no competing interests exist.

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