

Original Research Article

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Antibiotic Resistance Pattern of the Blood Culture Isolates of Adult Sepsis Patients from a Rural Based Tertiary Care and Teaching Hospital, Piparia, Vadodara, India

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ABSTRACT

The rapid emergence of resistance against the antibiotics by bacteria poses a threat to the health and health benefits that can be obtained by the use of antibiotics. This problem is worldwide and it reflects on the overuse of these drugs and lack of development of new drugs for combating this situation. Moreover infections due to such resistant bacteria add to the cost of healthcare in any nation. Thus performing antibiotic susceptibility and accordingly choosing the right antibiotic in each case to be treated invasive infections or non-invasive infections will prevent unjustified use of antibiotics as well as helps generate data regarding resistant bacteria and development of policies to prevent antibiotic resistance. A total of 673 blood cultures were performed from 743 clinically diagnosed sepsis patients. A total of 339 isolates were obtained from 330 (49.18%) blood culture positive samples. Of the 339 isolates, 51.32% (174/339) were Gram negative bacilli, 38.64% (131/339) were Gram positive bacteria and 10.02% (34/339) *Candida species*. All the bacterial isolates, gram positive as well as gram negative were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion method. Least resistance was observed against cabapenems i.e. 21.8% with 10.26% against Imipenem and 11.54% against Ertapenem and Meropenem with *Acinetobacter spp.* showing the highest resistance i.e. 29.63% whereas the most was seen against Cefuroxime, Cefotaxime and Ceftazidime.

Keywords

Sepsis, Blood culture, Antibiotics, Resistance

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Introduction

The rapid emergence of resistance against the antibiotics by bacteria poses a threat to the health and health benefits that can be obtained by the use of antibiotics. This problem is worldwide and it reflects on the overuse of these drugs and lack of development of new drugs for combating this situation. Moreover infections due to such resistant bacteria add to the cost of healthcare in any nation (Ventola, 2015). Thus performing antibiotic

susceptibility and accordingly choosing the right antibiotic in each case to be treated invasive infections or non-invasive infections will prevent unjustified use of antibiotics as well as helps generate data regarding resistant bacteria and development of policies to prevent antibiotic resistance.

This study was aimed at determining the antibiotic resistance pattern of the blood culture isolates of clinically diagnosed sepsis patients in our hospital. Also the same can be

utilised for treatment of the patients already diagnosed and also to choose empirical treatment for the patients suspected having sepsis.

Materials and Methods

A total of 673 blood cultures were performed from 743 clinically diagnosed sepsis patients. Of these, 330 samples showed growth with 339 isolates – with 51.32% (174/339) Gram negative bacilli, 38.64% (131/339) Gram positive bacteria and 10.02% (34/339) *Candida species*. Both the gram negative bacilli and gram positive cocci were subjected to antibiotic susceptibility testing by Kirby-Bauer Method (Collee *et al.*, 14th edn, Betty A. Forbes *et al.*, 12th edn, Koneman *et al.*, 6th edn.) according to CLSI guidelines (CLSI, 2011).

AST was performed by modified Kirby-Bauer disk diffusion method. A well isolated colony or morphologically similar colonies were picked up with the help of sterile wire and mixed with sterile normal saline to prepare an inoculum of 0.5 McFarland. The mixture was then vortexed for homogenous turbidity.

A sterile swab was dipped into the prepared inoculum and it was ensured that extra solution was drained by rolling the swab against the wall of the tube. Using this swab, a lawn culture was prepared on Mueller Hinton Agar (HiMedia). By rotating the plate thrice at 60° it was ensured that the whole surface is covered and uniform lawn culture is made. The antibiotic discs were placed on the plate within 15 minutes of inoculation and then the plates were incubated for 24 hours at 37°C. The following antibiotic discs were used depending upon the isolate type:

For Gram positive cocci

Penicillin G (10 units), Erythromycin (15µg), Levofloxacin (5µg), Gentamicin (10µg),

Cotrimoxazole (25µg i.e.1.25/23.75), Vancomycin (30µg), Linezolid (15µg) and Doxycycline (30µg) were tested.

For Gram negative bacilli

Imipenem (10µg), Amikacin (30µg), Gentamicin (10µg), Cefepime (30µg), Cefuroxime (30µg), Ceftazidime (30µg), Cefotaxime (30µg) Ciprofloxacin (5µg), Amoxicillin + Clavulanic Acid (30µg i.e. 20/10 µg) and Cotrimoxazole (25µg i.e. 1.25/23.75)) were tested.

For *Pseudomonas spp.*

Imipenem (10µg), Piperacillin (100µg), Piperacillin + Tazobactam (100/10µg), Ceftazidime (30µg), Amikacin (30µg), Gentamicin (10µg) and Cefipime (30µg) were tested.

The observations were made by measuring the sizes of zones of inhibition around each disc using the zone scale (HiMedia) for each isolated organism. These zone sizes were then interpreted as Susceptible/Sensitive (S), Resistant (R) and Intermediate (I) according to the manufacturer's zone size interpretative chart which is as per the CLSI guidelines.

VRSA detection: (CLSI, 2012 and 2013)

As per CLSI, disc diffusion testing is not a reliable method for detection of vancomycin resistance. Hence MIC should be performed for all isolates which show no zone of inhibition around the disc before reporting it as resistant. Thus for detection of VRSA, MIC was performed using Vancomycin Ezy MIC strip (HiMedia) according to the manufacturer's instructions.

For this the colonies isolated and identified as *Staphylococcus aureus* were picked and inoculum of 0.5 McFarland turbidity was prepared. A lawn culture was prepared using

the above inoculum. With the help of the applicator stick provided with the package, the strip was placed on the centre of the plate and the plate was incubated for 18-24 hours. The control strains used: *Enterococcus faecalis* ATCC29212– Susceptible *E. faecalis* ATCC51299– Resistant as well as *S. aureus* ATCC 25923

The MIC was read where the ellipse intersected the MIC scale on the strip. These strips have a continuous gradient and thus MIC values may fall “in-between” two fold dilutions. In such cases the values were rounded up to the next two fold dilution before categorization as per the manufacturer’s instructions.

According to CLSI the MIC values of $\leq 2\mu\text{g/ml}$ as well as $\leq 4\mu\text{g/ml}$ should be considered as susceptible while those with $\geq 8\mu\text{g/ml}$ should be sent to the reference laboratory for confirmation before reporting as resistant.

Results and Discussion

The antibiotic resistance pattern of the major gram negative bacilli isolated from blood excluding *Pseudomonas species*, *Proteus species*, *Citrobacter freundii* and unidentified GNB is shown (in the Table 1 and Chart 1) below i.e. (n=174-18=156). Least resistance was observed against cabapenems i.e. 21.8% with 10.26% against Imipenem and 11.54% against Ertapenem and Meropenem with *Acinetobacter spp.* showing the highest resistance i.e. 29.63% whereas the most was seen against Cefuroxime, Cefotaxime and Ceftazidime.

A total of 89.39%, 55.56% and 45.83% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance to amoxicillin-clavulanic acid. A total of 57.58%, 25.93% and 22.92%

of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance against Amikacin. Moreover, 93.94%, 96.30% and 87.50% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance to Cefuroxime and almost a similar percentage of resistance against Cefotaxime. The *Salmonella* species showed 100% susceptibility to Imipenem, Ertapenem/Meropenem, Gentamicin, Amikacin, Cefotaxime, Ceftazidime, Cefoxitin and Cefepime; but exhibited 20% resistance towards Cefuroxime and 6.66% resistance to Amoxicillin-Clavulanic acid and Ciprofloxacin.

Thus overall, least resistance was noted against imipenem (10.26%) and ertapenem/meropenem (11.54%), slightly higher against cefepime (44.87%), cotrimoxazole (42.31%), amikacin (35.90%) whereas highest was seen against cefuroxime (85.26%) followed by cefotaxime (79.49%), ceftazidime (77.56%), ciprofloxacin (74.36%) and gentamicin (68.59%) as shown in the Chart 1

Pseudomonas spp. from blood showed much higher resistance to most of the antibiotics as compared to all other gram negative bacilli with as high as 50% resistance to Imipenem and Ertapenem/Meropenem both as shown in the Chart 2

A total of 38.64% (131/339) isolates were Gram positive bacteria including 2 Gram positive bacilli. Thus a total of 38.05% (129/339) were Gram positive cocci and of which 39.53% (51/129) were *S. aureus*, 37.98% (49/129) *CoNS*, 19.37% (25/129) *Enterococcus species* and 3.1% (4/129) were *Streptococcus pyogenes*. As shown (in the Chart 3) the maximum resistance was observed against Penicillin and Erythromycin whereas least was against Linezolid and Doxycycline. All the isolates showed 100% susceptibility to Vancomycin.

Table.1 Antibiotic resistance pattern of major Gram negative isolates of blood (%)

Blood Cultures Isolates (n=174-18=156)	IPM	AMC	AK	GEN	CPM	CIP	CXM	CTX	COT	CAZ	CX	ERT/ MRP
<i>Klebsiella spp.</i> (n=66)	7.58	89.39	57.58	84.85	65.15	78.79	93.94	92.42	69.70	93.94	22.73	9.09
<i>E. coli</i> (n=48)	6.25	45.83	22.92	64.58	31.25	83.33	87.50	85.42	20.83	79.17	37.50	6.25
<i>Acinetobacter spp.</i> (n=27)	29.63	55.56	25.93	74.07	44.44	85.19	96.30	81.48	37.04	77.78	40.74	33.33
<i>Salmonella species</i> (n=15)	0.00	6.67	0.00	0.00	0.00	6.67	20.00	0.00	0.00	0.00	0.00	0.00

Chart.1 Total percentage of resistance exhibited by major Gram negative bacilli

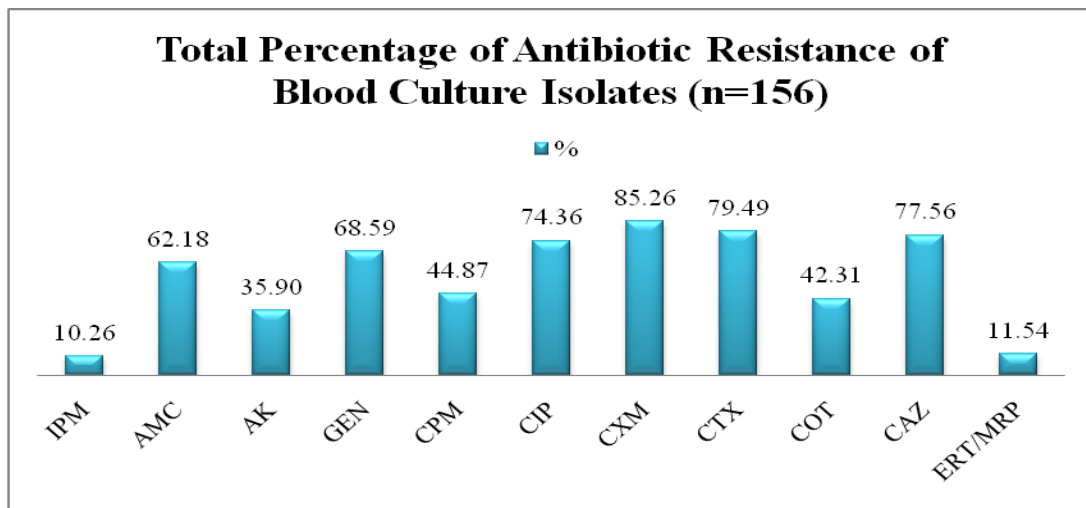


Chart.2 Antibiotic Resistance Pattern of *Pseudomonas spp.* (n=14)

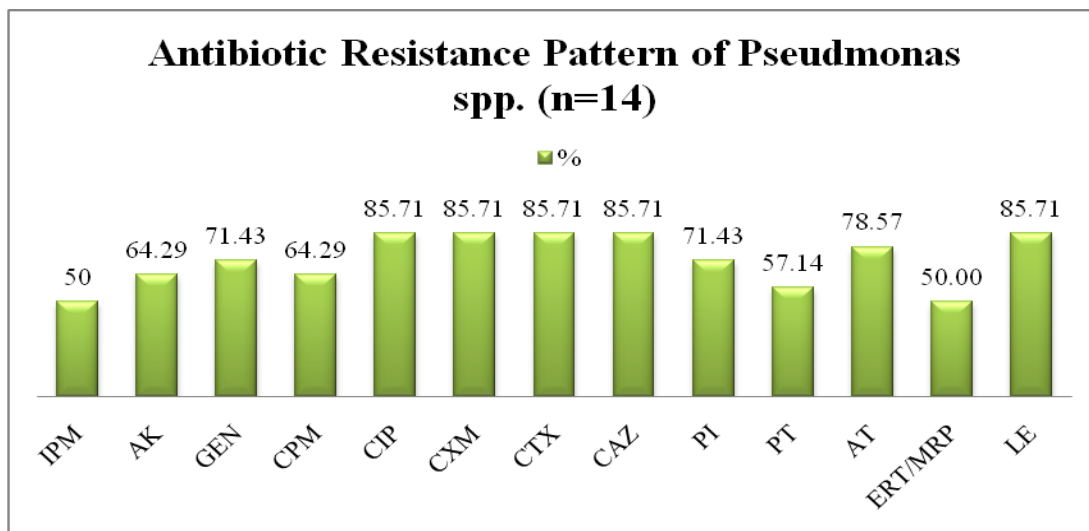


Chart.3 Antibiotic resistance pattern of Gram positive cocci from blood culture

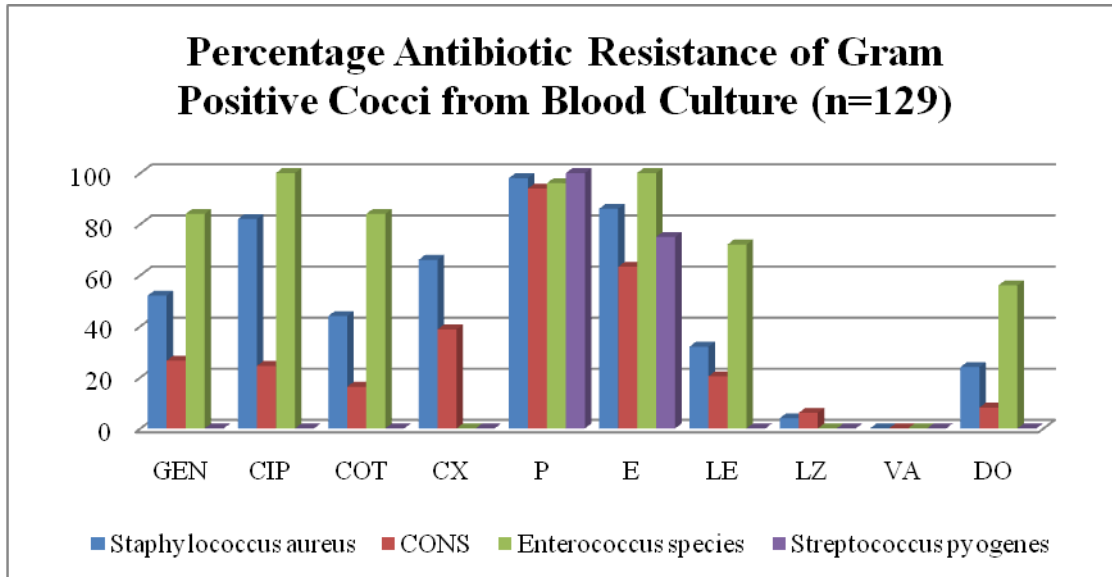
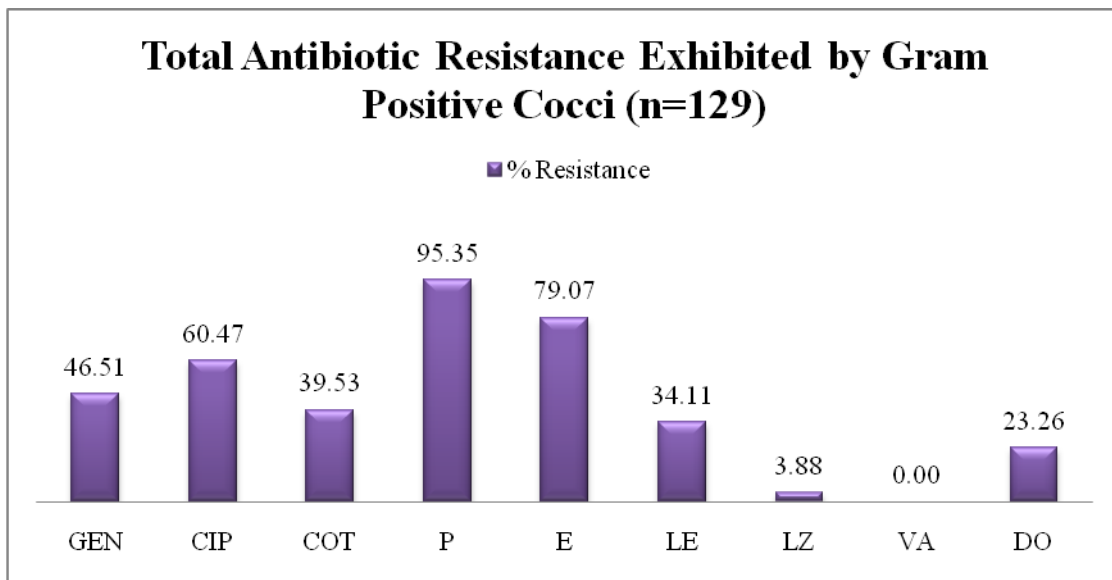


Chart.4 Total percentage of antibiotic resistance exhibited by Gram positive cocci (n=129)



Moreover, the maximum resistance against most of the antibiotics was shown by *Enterococcus species* isolates.

Total of 52.94% (27/51) *S. aureus* were found to be *MRSA* and 34.69% (17/49) were found to be *MRCONS*. The antibiotic resistance pattern of the gram positive culture isolates of blood is as shown in the Chart 3.

The overall resistance against antibiotics tested for gram positive cocci reveals 0% resistance towards vancomycin and 95.35% towards penicillin with varying resistance towards other antibiotics tested as shown in the Chart 4.

Thus overall least resistance was observed against carbapenems with *Acinetobacter spp.*

showing the highest resistance i.e. 29.63%. A total of 89.39%, 55.56% and 45.83% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance to amoxicillin/clavulanic acid. A total of 57.58%, 25.93% and 22.92% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance against Amikacin. Moreover, 93.94%, 96.30% and 87.50% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance to Cefuroxime and almost a similar percentage of resistance against Cefotaxime. Thus higher percentage of resistance against amoxicillin/clavulanic acid and 3rd generation cephalosporins is comparable to the findings of Oza *et al.*, (2016) from Surendranagar and Kante *et al.*, (2014) from AP, India. Ugas *et al.*, (2016) reports a case of septic shock with acute respiratory distress syndrome due to *Salmonella typhi* from Oklahoma, USA, which was found to be susceptible to ceftriaxone, cotrimoxazole and ciprofloxacin. Another case report from New Delhi, India by Randhawa VS *et al.*, 2007, reports *Salmonella paratyphiA*, susceptible to Cefotaxime, Ciprofloxacin, Gentamicin, Amikacin, Cotrimoxazole which is similar to the findings of this study.

No resistance was observed against vancomycin and least towards linezolid (3.88%) and doxycycline (23.26%) whereas 97.42% against Penicillin and 85.57% against Erythromycin with *Enterococcus spp.* showing the maximum resistance. These findings are again comparable to the findings of Oza *et al.*, (2016) who report no resistance against vancomycin, linezolid and teicoplanin and also higher resistance by *Enterococcus spp.* towards penicillin. However, findings in regards to Penicillin and Erythromycin are in striking contrast to 100% susceptibility reported by Kante *et al.*, (2014). Also resistance against ciprofloxacin was noted as 60.47% and 74.36% by GNB and GPC respectively. This is in contrast to the findings

of Kumalo *et al.*, (2016) who report ciprofloxacin to be 87.5% susceptible and as an effective antibiotic in their setup.

Thus it is important for any setup to perform antibiotic susceptibility testing of the isolates obtained which helps to generate data regarding resistance pattern of the isolates in any setup. We also saw an increased resistance against most of the antibiotics tested in our setup similar to the findings of the other studies. In our setup least resistance was seen against Imipenem amongst gram negative bacilli and against vancomycin amongst gram positive cocci rendering these as choice of treatment.

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