

Original Research Article

Antibiotic resistance pattern of bacteria isolated from various clinical specimens: an eastern Indian study

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ABSTRACT

Background: Resistance to antibiotics is an extremely common phenomenon in bacteria isolated from clinical material. This is a serious threat to patient care all over the world. In India, antibiotic resistance has far reaching public health consequences. In this observational study, we aimed to generate data on the prevailing pattern of antibiotic resistance from Eastern India.

Methods: This was a hospital based study involving both indoor and outdoor patients. Patients with history of antibiotic use in the past 3 months were excluded. The clinical specimens (blood, urine, pus etc.) were incubated for up to 7 days under aerobic conditions before declaring them as negative. Antibiotic sensitivity was tested by modified Kirby-Bauer disc diffusion method according to CLSI guidelines.

Results: There were a total of 93 specimens with the majority being blood culture (n=43) and urine (n=33). Majority (n=57; 61.3%) of isolated organisms were gram negative with *E. coli* predominating (n=36). Of the gram-positive isolates, *Staphylococcus* predominated (32 out of 36). 100% of isolates from urine were gram negative while for blood, 60% of the isolates were gram positive. Resistance to penicillin group and cotrimoxazole was up to 100% in certain species. For carbapenem group, resistance varied from 17 to 75%. Resistance to aminoglycosides was 75% in *Pseudomonas* and 85% in *Klebsiella*.

Conclusions: Our study has demonstrated very high levels of resistance to different common antibiotics in different classes of bacteria. Such data can be used for antibiotic stewardship and also to formulate antibiotic use protocols.

Keywords: Antibiotic resistance, Bacteria, Aminoglycosides

INTRODUCTION

Resistance of bacteria to antimicrobial agents is an imminent threat to patient management all over the world. This issue has plagued policy makers and clinicians everywhere but there seems to be no simple way of circumventing the problem.¹ Rapidly rising antibiotic resistance is a challenge to comprehensive patient care in all branches of medical science. The interaction between various clinical bacteria and the antimicrobial agents is a complex issue involving the prokaryotic adaptive mechanisms and genetic changes.¹

This complex interaction must be studied in depth in order to achieve a sustainable and effective solution to the looming threat of antibiotic resistance.

Earlier, the problem of antibiotic resistance was primarily a concern for nosocomial infections. But now, even community acquired infections are caused by organisms with high levels of antibiotic resistance.¹ As a recent report demonstrates, such multi-drug resistant community acquired infections can be a cause of significant morbidity.² Earlier, such drug resistant organisms were said to infect mainly patients with identifiable risk factors or profound immunosuppression. But now, reports are

showing such infections in seemingly normal healthy persons. Also, such drug-resistant infections may complicate the newly emerging infectious diseases. For example, influenza epidemics now are sometimes reported to be complicated by superadded infection with drug-resistant bacteria.³

The issue of drug resistance in clinical bacteria is such a vital threat that the UN held a special assembly in 2016 to address only this issue.⁴ In that assembly, the issue was said to be of as much importance as climate change and it was deemed to require a global response.⁴

The pattern of antibiotic resistance in bacteria in India shows a trend similar to the global epidemic. India is one of the highest consumers of antibiotic drugs in the world.⁵ Studies from different parts of India have consistently documented an ominously rising level of resistance to all the common antibiotics.⁵ Over the last decade, published data have revealed doubling or even tripling of the rate of antibiotic resistance for almost all groups of clinically important pathogens.

However, data from Eastern India are comparatively less compared to the other parts of the country. In this study, we aimed to generate data on the prevalence of antibiotic resistance from a sample population of this part of the country. As antibiotic stewardship is becoming an important issue for all health care systems, such local data is of vital importance in deciding on treatment protocols.

METHODS

This observational study was done in a tertiary care hospital of Eastern India from July 2015 to September 2016. It was done in the general medicine indoor ward and outdoor departments. Adult patients with suitable clinical features suggestive of infection (dysuria, high fever >100°F, non-healing wound >1 week etc.) were first selected for the study. Then, they were explained about the study protocol in details and informed consent was obtained for microbiological examination of their clinical specimen, as suitable. Exclusion criteria for the study included anyone receiving antibiotics in the past 3 months, anyone with known foreign bodies in situ like mechanical heart valve, prosthetic joints etc. or anyone who had had any invasive procedure like endoscopy or dental surgery in the past three months. A flow-chart for study subject selection is shown in Figure 1.

The clinical samples were obtained aseptically. Only one sample (blood, urine or pus) was taken from each patient. For blood culture, inoculation of the culture bottle was done at the bedside. All samples were sent to the microbiology laboratory within one hour, avoiding exposure to sunlight, excess heat or cold during transport. The culture was done for up to 7 days under aerobic conditions before declaring the specimen as negative. Inoculation was done both in agar plate and McConkey

agar. If an organism was grown, it was further subjected to suitable biochemical tests for identification. If more than two organisms were isolated from a particular clinical specimen, it was taken to be contaminated and rejected.

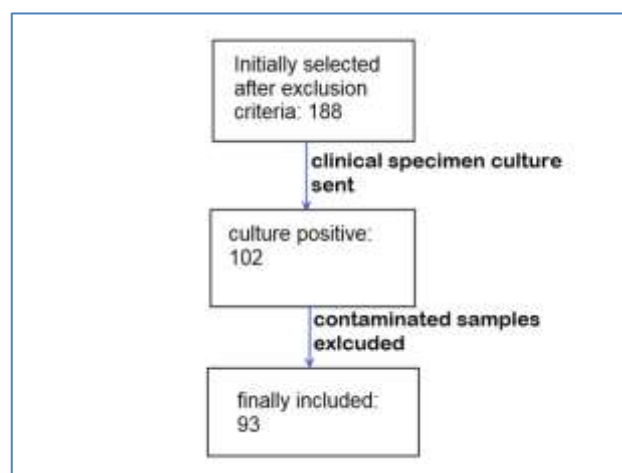


Figure 1: Showing the process of selection of study subjects.

After isolation of the organisms, they were tested for antibiotic sensitivity by modified Kirby-Bauer disc diffusion methods, following the CLSI guidelines.⁶ In consultation with microbiologist, the resistance patterns were reported as “sensitive” or “resistant”. The “intermediate resistant” term was avoided. The antibiotics tested included beta-lactam group (penicillins and cephalosporins), aminoglycosides, macrolides, clindamycin, glycopeptides, colistin, carbapenem, tigecycline, fluoroquinolones, cotrimoxazole and nitrofurantoin. However, some modification was done based on the organism. For example, for gram negatives, glycopeptides like vancomycin (which are specific for gram positives) were not tested.

Statistical analysis was done using SPSS ver. 20. Proportions and percentages were used to describe the resistance pattern.

RESULTS

The average age of the patients was 48.2± 17.5 years and the male: female ratio was 48: 45.

In this study, we had a total of 93 clinical specimens: 33 urine, 43 blood culture, 8 sputum and the rest miscellaneous including wound swab, central line catheter tip and pus from body cavities like empyema. The isolated organisms are shown in Figure 2.

61.3% of the isolated organisms (n=57) were gram negative with 36 (63%) *Escherichia coli* and 13 (22.8%) *Klebsiella*. Of the gram positive organisms (n=36), 16 were *Staphylococcus aureus* and 4 were *Enterococci*.

Table 1 shows the organisms isolated from different clinical specimens. It is seen that all isolates from urine were gram negative while from sputum and wound swab,

the majority were gram positive isolates. For blood culture, the outcome was more balanced (gram positive: negative= 26:17).

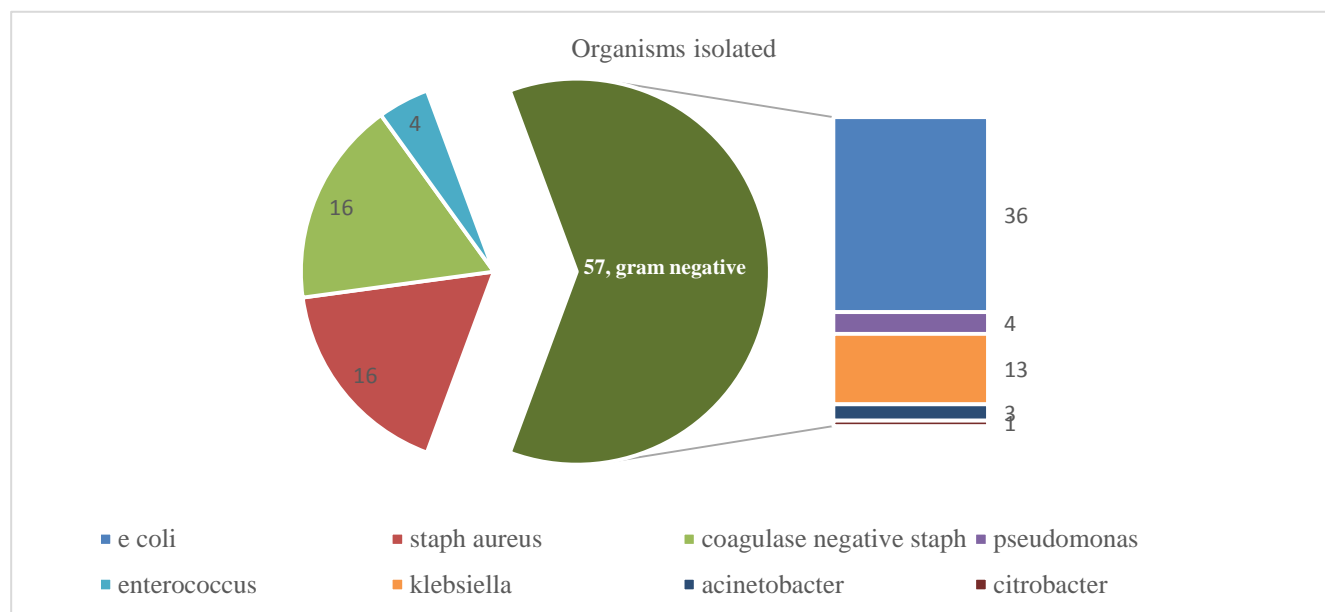


Figure 2: Pie chart showing the organisms isolated in our study.

Table 1: Table showing the bacteria isolated from different clinical specimens.

Clinical material	Organism isolated	
	Gram positive	Gram negative
Urine (n=33)	0	33
Blood (n=43)	26	17
Sputum (n=8)	5	3
Wound swab (n=6)	5	1
Others (n=3)	0	3

Table 2: Table showing the percentage of resistance to different classes of antimicrobial agents.

Bacteria	Penicillin and its congeners	3 rd gen CPN	4 th gen CPN	CP	FQ	AG	M	TC	GP	LZ	CM
Gram positive											
<i>Staphylococcus aureus</i>	94	75	88	50	69	69	75	-	6	6	81
<i>Coagulase negative staphylococcus</i>	100	81	81	56	62	50	75	--	12	6	87
<i>Enterococci</i>	100	75	100	75	100	50	100	--	0	0	75
Gram negative											
<i>E.coli</i>	97	64	94	17	22	14	--	89	--	--	94
<i>Klebsiella</i>	100	92	85	62	92	85	--	77	--	--	77
<i>Acinetobacter</i>	100	100	100	33	100	33	--	66	--	--	100
<i>Pseudomonas</i>	100	50	50	25	50	75	--	50	--	--	75

CPN: Cephalosporin; CP: carbapenem; FQ: fluoroquinolones; AG: aminoglycosides; M: macrolide; TC: tetracycline; GP: glycopeptides; LZ: linezolid; CM: cotrimoxazole (Septran).

Table 2 shows the pattern of resistance to different classes of antibiotics for the different classes of bacteria. As seen here, resistance to penicillin group of drugs

(penicillin, amoxicillin, ampicillin, oxacillin etc.) was 94—100% for both gram positive and gram negative species. For carbapenem class, *Klebsiella* showed 62%

resistance and *Enterococci* had 75% resistance. In the gram positive group, macrolide resistance varied from 75-100%. Vancomycin and linezolid resistance were very rare. Cotrimoxazole resistance varied from 75—100% for different species. 85% of the *Klebsiella* (11 out of 13) were resistant to aminoglycosides. For pseudomonas, this was 75%.

DISCUSSION

Our observational study has shown very high levels of resistance to antimicrobial agents belonging to different classes, for both gram positive and gram negative bacteria.

Antibiotic resistance of bacteria is a significant threat all over the world. But for developing countries like India this is an even greater public health problem.⁷ This is because India has one of the highest burden of bacterial diseases in the world and thus, antibiotics have a significant role in reducing mortality and morbidity in the country.⁷ But the WHO survey on antibiotic use in India has revealed a disturbing picture. It has shown that most people in India have a very high frequency of antibiotic use for unnecessary purposes and in most cases, these were prescribed from the medical institution.^{7,8} Such practises have contributed to an ominous trend of rising antibiotic resistance in the country.

Recent studies have shown multi-drug resistant organisms emerging from all parts of India.⁹ This trend has been documented for both gram positive and gram negative bacteriae.^{9,10} A ten year study from North India has recently demonstrated rapid rise in antibiotic resistance in clinical isolates (*E. coli* and *Klebsiella*) over this ten year period.¹⁰ Bacteria demonstrated resistance not only to the older antibiotics, but also to the newly introduced ones like tigecycline, very rapidly. The percentage of organisms resistant to carbapenem increased from 2.4 to 52 over a 7 year period for *Klebsiella* in this study.¹⁰ Another study from South India has shown a similar trend.¹¹ Carbapenem resistance in pseudomonas increased from 36% to 65% in one year and ceftriaxone resistance in *E. coli* was 97% in this study. In our study, 3rd generation cephalosporin resistance in *E. coli* was 64% but for *Klebsiella* and *Acinetobacter*, it was close to 100% (Table 2).

From Eastern India, the data is comparatively less. A very recent study from West Bengal has shown a rising trend of resistance to beta lactams, tetracyclines and cephalosporins in common urinary pathogens of *E. coli* and *Klebsiella*.¹² A large number of subjects in this study were outpatient department attendees and thus, the resistance pattern represented the prevalence of antibiotic resistance in the community. Between 2008 and 13, resistance to 3rd and 4th generation Cephalosporins increased from 20 to almost 50%.¹² In our study, for *E. coli* and *Klebsiella*, resistance to 3rd and 4th generation cephalosporins varied from 64 to 94%.

There are many reasons for this rapid rise of antibiotic resistance in India. One reason is the prevailing trend of prescribing antibiotics for different symptoms like fever, where antibiotics are not indicated.¹³ This trend is present in both urban and rural settings and thus, antibiotic resistant bacteria have been isolated from even remote localities.¹³ In a recent study from south India, done in a completely rural set up, Kousalya et al have demonstrated significant levels of resistance to 3rd generation cephalosporins and aminoglycosides in common respiratory pathogens.¹⁴ Another potential cause is the practice by Indian pharmaceutical companies of manufacturing and marketing antibiotic combination tablets or capsules.¹⁵ Many of these are completely irrational and only serve to generate antibiotic resistance. In the 5 years between 2006 and 2011, antibiotic consumption in India doubled; but this increased consumption was not always for approved or clinically relevant indications.¹⁵ Finally, in many cases patients in India obtain antibiotics without a prescription.¹⁵ Such spurious use is likely to result in inadequate dosage, which only helps to foster further resistance.

Increasing antibiotic resistance in bacteria will mean increased use of last line antimicrobial agents. These have more side effects and much increased costs. A rough estimate of the economic burden of antimicrobial resistance shows that for resistant *Staphylococcus aureus*, the excess cost can vary between 700 and 30000 USD.¹⁶ For resistant Enterobacteriaceae like *E. coli*, this excess cost can be up to 4900 USD per patient year. For a country like India, where most of the healthcare spending is out-of-pocket, such increased cost will often mean further increase in poverty.

Thus, urgent steps must be taken to address this issue at all levels. Since the health sector in India is a conglomeration of government, charitable and corporate sectors, coordination will be very difficult. But actions at all levels, social, political, professional and scientific are needed to push this agenda forward.

Our study is limited by the small number of samples, lack of genetic testing for the mechanism of antibiotic resistance and inclusion of only adults in the study population. But still, our study shows that the prevalence of antibiotic resistance in Eastern India is similar to other parts of India. Larger multicentric studies are needed to find the trend of development of resistance. This will help in formulating antibiotic use protocols.

CONCLUSION

Antibiotic resistance in bacteria is a major threat for clinicians. Judicious use of antibiotics and frequent surveillance are needed to curb this threat and preserve the antibiotics for the future.

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