

Original Research Article

Multidrug resistant, extensively drug resistant and pan drug resistant gram negative bacteria at a tertiary care centre in Bhubaneswar

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ABSTRACT

Background: Multidrug resistance has emerged as a challenge in health care settings. Again increasing prevalence of multidrug resistant (MDR), extensively drug resistant (XDR) and pan drug resistant (PDR) gram negative bacteria is making the condition more critical because of limited options of antibiotics, increasing morbidity, mortality and hospital stay of the patients. The present study is carried out with an aim to estimate the prevalence of MDR, XDR, PDR gram negative bacteria in a tertiary care hospital.

Methods: Total of 912 gram negative bacterial isolates obtained from various samples of indoor patients in a tertiary care hospital, were studied over a period of six months. The bacteria were identified by conventional methods. Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method. Minimum inhibitory concentration (MIC) of antibiotics for the resistant isolates were detected by Vitek-2 automated method. MDR, XDR and PDR were determined according to the definitions suggested by European Centre for Disease Prevention and Control (ECDC), and Centers for Disease Control and Prevention (CDC). Prevalence of extended spectrum beta lactamase (ESBL) producers was estimated.

Results: Out of 912 isolates, prevalence of MDR, XDR and PDR were 66.12%, 34.32% and 0.98% respectively. Prevalence of MDR and XDR were higher in ICUs than clinical wards ($p < 0.0001$). Prevalence of ESBL producers was 48.4%.

Conclusions: The study highlights increased prevalence of multidrug resistant and extensively drug resistant strains in our hospital. Stringent surveillance, proper implementation of hospital infection control practices and antimicrobial stewardship will help in limiting the emergence and spread of drug resistant strains.

Keywords: Gram negative bacilli, Multi drug resistant, Extensively drug resistant, Pan drug resistant

INTRODUCTION

Discovery of antimicrobials is one of the most important milestones achieved in the history of medical science. They behaved as wonder drugs in the treatment of infectious diseases till the advent of antimicrobial resistance. The burden of drug resistance has increased so

much that it has emerged as a major challenge of 21st century in health care system.¹ In developing countries like India the problem of antibiotic resistance is quite higher compared to developed countries.² Recently, the emergence of multi drug resistant strains of bacteria has been considered as a major public health problem by World Health Organisation (WHO).³ Again the

prevalence of multidrug resistant gram negative bacilli is increasing significantly.⁴ Over last two decades the infections caused by these multidrug resistant gram negative strains have increased morbidity, mortality and the duration of hospital stay, particularly in developing countries.⁵ Among gram negative bacteria, members of *Enterobacteriaceae* such as *Escherichia coli*, *Klebsiella spp.*, *Enterobacter spp.*, *Proteus spp.* and among non lactose fermenters, *Pseudomonas spp.*, *Acinetobacter spp.*, have been identified as predominant cause of multidrug resistant bacterial infections.⁶⁻⁸ European Centre for Disease Control (ECDC) and Centre for Disease Control and Prevention (CDC), Atlanta have proposed standardised definitions for the multidrug-resistant (MDR), extensively drug resistant (XDR), and pan drug resistant (PDR) bacteria.⁹ Multidrug resistant (MDR) was defined as acquired non susceptibility to at least one agent in three or more antimicrobial categories as per guidelines. Extensively drug resistant (XDR) was defined as non susceptibility to at least one agent in all but two or fewer antimicrobial categories listed and pandrug resistant (PDR) was defined as non susceptibility to all agents in all antimicrobial categories.⁹

Various studies have found out that there are differences in resistance pattern of the bacteria depending on different regions even different institutions.^{10,11} Hence this study was carried out with an aim to know the prevalence of MDR, XDR and PDR gram negative bacteria at a tertiary care hospital in Odisha and to help implementing an effective antibiotic policy.

METHODS

This was a cross sectional study carried out between 1st of January to 30th of June 2017 at Kalinga Institute of Medical Sciences (KIMS), a tertiary care teaching hospital in Bhubaneswar, Odisha.

Inclusion and exclusion criteria

A total of 912 non repetitive and consecutive bacterial strains isolated from various samples (respiratory samples, urine, blood, body fluids, wound swab, pus, tissue samples etc.) of indoor patient of wards and different ICUs were included in the study. Clinical samples from outdoor patients were not included.

The bacterial strains were identified by conventional phenotypic methods, using gram's staining and different biochemical reactions.¹² Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method on Mueller-Hinton agar and results were interpreted as per CLSI guidelines.^{13,14} Dehydrated media and antibiotic discs procured from Hi-Media (Mumbai) were used. In case of bacterial isolates those showed resistance to all antibiotics by disc diffusion method, minimum inhibitory concentration (MIC) value was determined for antibiotics like colistin, polymixin B, tigecycline by automated method (Vitek2 compact; bioMerieux, France). Intrinsic

resistance of bacteria for concerned antibiotics was considered while detecting resistant strains. MDR, XDR and PDR bacterial strains were detected according to guidelines proposed by ECDC and CDC.⁹ Extended spectrum beta lactamase (ESBL) producing strains were detected amongst members of *Enterobacteriaceae* by disc diffusion method using ceftazidime (30µg), cefotaxime (30µg), ceftazidime plus clavulanic acid (30/10µg) and cefotaxime plus clavulanic acid (30/10µg) combination. For a bacterial isolate, an increase in zone diameter by ≥ 5 mm with ceftazidime and clavulanic acid combination disc in comparison to ceftazidime disc alone and or ≥ 5 mm increase in the zone diameter of cefotaxime/clavulanic acid disc and that of cefotaxime disc alone was considered to be phenotypic confirmatory test for that strain.¹⁴ For quality control purpose *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 were taken as control strains.¹⁴

The data was entered in Microsoft excel 2010 version and compared. Statistical significance was calculated using chi-square test. P<0.05 was considered to be statistically significant.

RESULTS

A total number of 912 gram negative bacterial isolates isolated from 784 various samples were studied. Out of these, 375 isolates were obtained from samples of intensive care units and 537 isolated were from samples of other clinical wards. Maximum number of isolates were obtained from urine samples (387/42.43%), followed by sputum (135/14.8%) and tracheal secretions (99/10.85%) (Table 1).

Table 1: Number of gram negative bacteria isolated from various clinical samples.

Clinical samples (number)	No. of GNB isolated N (%)
Urine (362)	387 (42.43)
Sputum (112)	135 (14.8)
Tracheal secretion (78)	99 (10.85)
Pus (74)	96 (10.53)
Blood (76)	96 (10.53)
Body fluid (6)	9 (0.99)
Stool (24)	24 (2.63)
Others (52)	66 (7.24)
Total sample (784)	n=912

Amongst 912 gram negative bacterial isolates most common was *Escherichia coli* (267/29.3%), followed by *Klebsiella spp.* (255/27.9%) and *Acinetobacter spp.* (141/15.5%) (Table 2). 48.4% (283/585) isolates were ESBL producers. Out of these, maximum number of ESBL producers were *Escherichia coli* isolates (154/57.6%) followed by *Klebsiella spp.* (111/43.5%) (Figure 1).

Table 2: Distribution of bacterial isolates in clinical samples (total no. of isolates n=912).

Sample	<i>E. coli</i>	<i>Klebsiella sp.</i>	<i>Enterobacter sp.</i>	<i>Citrobacter sp.</i>	<i>Proteus sp.</i>	<i>Pseudomonas sp.</i>	<i>Acinetobacter sp.</i>	Others
Urine	201	81	9	9	24	24	21	18
Pus	9	33	6	3	---	15	21	9
Blood	9	30	---	---	---	9	18	30
Tracheal secretion	3	27	---	---	3	21	39	6
Body fluid	3	6	---	---	---	---	---	---
Stool	24	---	---	---	---	---	---	---
Sputum	3	75	3	---	---	27	27	---
Others	15	3	6	---	---	21	15	6
Total no.	267	255	24	12	27	117	141	69
(%)	(29.3)	(27.9)	(2.63)	(1.31)	(2.96)	(12.82)	(15.5)	(7.6)
ESBL producers (%)	154 (57.6)	111 (43.5)	9 (37.5)	3 (25)	6 (22.2)	---	---	11 (16)

Table 3: Distribution of MDR, XDR and PDR isolates in ICUs and clinical wards.

Types of isolates	ICUs (total no. of isolates = 375) N (%)	Clinical wards (total no. of isolates = 537) N (%)	Total	P value
MDR	281 (75)	322 (60)	603	<0.0001
XDR	171 (45.6)	142 (26.4)	313	<0.0001
PDR	6 (1.6)	3 (0.56)	9	0.22

Table 4: Prevalence of MDR, XDR& PDR strains among gram negative bacterial isolates.

Bacteria	Total numbers	MDR No. N (%)	XDR No. N (%)	PDR No. N (%)
<i>E. coli</i>	267	187 (70.04)	51 (19.1)	0
<i>Klebsiella sp.</i>	255	181 (71)	147 (57.65)	1 (0.4)
<i>Enterobacter sp.</i>	24	15 (62.5)	9 (37.5)	0
<i>Citrobacter sp.</i>	12	7 (58.33)	0	0
<i>Proteus sp.</i>	27	11 (40.74)	5 (18.52)	2 (7.4)
<i>Pseudomonas sp.</i>	117	69 (59)	21 (18)	2 (1.71)
<i>Acinetobacter sp.</i>	141	101 (71.63)	71 (50.35)	4 (2.84)
Others	69	32 (46.37)	9 (13.04)	0
Total	912	603	313	9

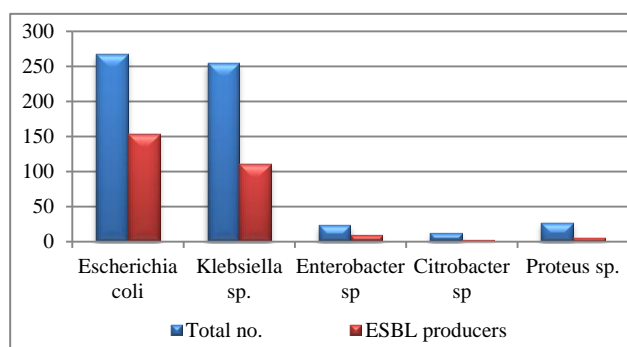


Figure 1: Distribution of ESBL producers among bacterial isolates.

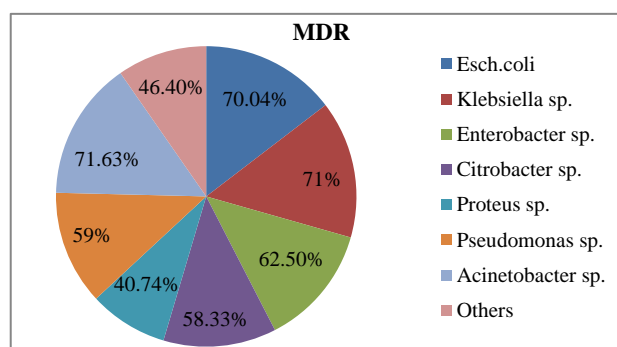


Figure 2: Prevalence of MDR strains among bacterial isolates.

Out of 912 bacterial isolates, 603(66.12%) were MDR strains, 313 (34.32%) were XDR and 9 (0.98%) were PDR strains. Number of MDR, XDR and PDR strains in intensive care units (ICUs) were 281 (75%), 171 (45.6%) and 6 (1.6%) respectively whereas the same in other clinical wards were 322 (60%), 142 (26.4%) and 3 (0.56%) respectively (Table 3).

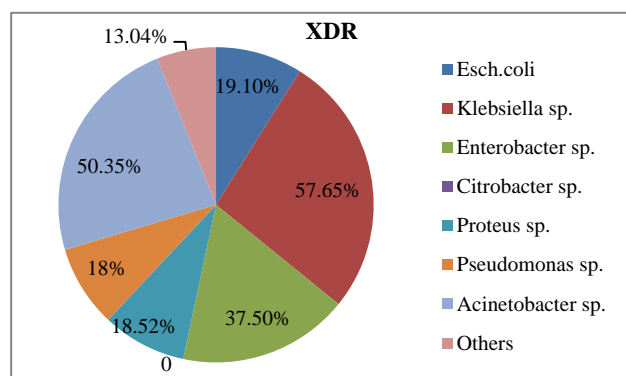


Figure 3: Prevalence of XDR strains among bacterial isolates.

In the present study most frequent MDR bacteria was *Acinetobacter* spp. 71.63% (101/141) followed by *Klebsiella* spp. 71% (181/255) and *E. coli* 70.04% (187/267) (Figure 2). Among XDR strains most common was *Klebsiella* spp. 57.65% (147/255) followed by *Acinetobacter* spp. 50.35% (71/141) Figure 3. Total of 9 PDR gram negative bacteria were observed in our study which consists of 4 (2.84%) *Acinetobacter* spp. strains, 2 (1.71%) *Pseudomonas* spp. strains (1.71%), 2 (7.4%) *Proteus* spp. strains, and 1 (0.4%) *Klebsiella* spp. strains (Table 4).

DISCUSSION

Increasing incidence of infections by MDR bacteria or superbugs has become a matter of grave concern in health care sectors as it accelerates both morbidity and mortality of the patients. Inappropriate use of antibiotics, rising number of immunocompromised patients and poor infection control practices contribute to emergence and further spread of MDR strains.¹⁵ According to a WHO survey, expanding global trade and tourism also help in spreading of drug resistant bacteria between continents. In addition lower pace of development of newer antibiotics in comparison to rapidly emerging MDR strains is making the condition more critical.^{16,17} Several studies have indicated increased incidence of multidrug resistance gram negative bacterial infections in hospitalised patients for last few years.^{18,19} For last few decades more emphasis is given on MDR gram positive bacteria compared to gram negative bacteria to combat the problem of drug resistance.¹⁷ This study was carried out with an aim to quantify the burden of MDR, XDR and PDR gram negative bacteria in our hospital.

In the present study urine was the predominant sample from which maximum number of gram negative bacteria (387/42.43%) were isolated (Table 1). Similar findings was seen in the study by Agyepong et al where maximum number of isolates (94/47%) were from urine sample.²⁰ It could be due to the fact that urine was the major sample in our study.

According to our study, most frequent gram negative bacteria was *E. coli* (267/29.3%) followed by *Klebsiella* spp. (255/27.9%) and *Acinetobacter* spp. (141/15.5%) (Table 2). Our finding correlates with some other studies like Agyepong et al (24.5%), Basak et al (35%), Folgori et al (67.6%), where most common gram negative bacteria isolated was *E. coli*.²⁰⁻²²

ESBL producing bacteria often contain MDR strains by exhibiting resistance to other classes of antibiotics in addition to penicillins, first, second and third generation cephalosporins and aztreonam.^{7,23} Prevalence of ESBL producers in our study was 48.4% (283/585). Different authors throughout India showed varying prevalence of ESBL producers like Basak et al (18.4%), Rodrigues et al (53%), Singhal et al (64%), Mathur et al (68%) etc.^{21,24-26}

In this study most common ESBL producer was *E. coli* (154/57.6%) followed by *Klebsiella* spp. (111/43.5%) (Table 2/Figure 1). Our finding correlated well with other studies like Rodrigues (65.8%), Dalela (73.5%) where *Escherichia coli* was the most common ESBL producer.^{24,27}

The prevalence of MDR, XDR and PDR strains in our study are 66.12% (603/912), 34.32% (313/912) and 0.98% (9/912) respectively. Study by Basak et al showed 33.5% MDR strains, 12.1% XDR and no PDR strains which is lower than our findings.²¹ Oliveria et al observed MDR prevalence to be 36%.¹⁰ Bhatt et al in their study found out prevalence of XDR and PDR strains as 8.1% (101/1240) and 0.9% (11/1240) respectively.²⁸ Adrizain et al showed MDR and XDR prevalence as 28.7% (86/299) and 4.7% (14/299) among paediatric patients from blood culture pathogens.²⁹

In the study done by Agyepong et al in Ghana, prevalence of MDR strains was 89.5% which is higher than prevalence of our study.²⁰ In the study by Bajpai et al percentage of MDR strains (75.8%) and PDR strains (2.1%) among uropathogens are higher than our observations for MDR and PDR strains whereas percentage of XDR strains (12%) is lower than our finding.³⁰

The higher prevalence in our study could be due to the fact that our institute is a tertiary care hospital where most of the patients are referred ones having prior exposure of antibiotics which could be a predictor factor for development of multi drug resistance.³¹

Percentage of isolation of MDR and XDR strains from ICU in our study were 75% (281/375) and 45.6% (171/375) which are higher than prevalence of MDR strains (60%, 322/537) and XDR strains (26.4%, 142/537) isolated from different clinical wards. This differences were also found to be statistically significant with $p < 0.0001$. Similar finding observed by Basak et al where percentage of MDR (52.2%) and XDR isolates (18.8%) from ICUs were more than MDR isolates (43.3%) and XDR isolates (16.2%) obtained from clinical wards.²¹ Oliveria et al also observed higher MDR isolation rate (67%) from ICUs than wards (33%).¹⁰ Higher prevalence of drug resistant strains in ICUs may be due to frequent exposure of critically ill patients to antibiotics, longer stay and interventional instrumentations such as use of ventilators, central lines, urinary catheters.^{31,32}

Prevalence of PDR in ICU (1.6%, 6/37) is more than that of clinical wards (0.56%, 3/537). This difference is not found to be statistically significant which could not be evaluated due to less number of PDR isolates (9) in our study.

Amongst all the MDR strains most common was *Acinetobacter* spp. 71.63% (101/141) followed by *Klebsiella* spp. 71% (181/255) and *E. coli* 70.04% (187/267). (Table 4, Figure 2). In the study by Agyepong et al most common MDR bacteria were *Acinetobacter* spp. (100%) and *Pseudomonas* sp. (100%) whereas Basak et al (31.6%) and Tohamy et al (38.6%) in their studies showed *E. coli* as the most common MDR strain.^{20,21,33}

Most common XDR strains were *Klebsiella* spp 57.65% (147/255) followed by *Acinetobacter* spp. 50.35% (71/141) (Table 4/Figure 3). In the study of Basak et al *Pseudomonas* spp. (32.2%) were the most common XDR strains.²¹

Out of total 9 PDR (0.98%) strains, four were *Acinetobacter* spp., two strains of *Pseudomonas* spp., two strains of *Proteus* spp. and one strain of *Klebsiella* spp. Our finding is similar to that of Bhatt et al where the prevalence of PDR was 0.9%.²⁸ In the study of Bajpai et al the prevalence of PDR (2.1%) is higher than our observation.³⁰ All these isolates were resistant to all antibiotics used as per CDC and ECDC recommendations including colistin, polymixin B and tigecycline. *Proteus* spp. strains were not tested against colistin and tigecycline as they are intrinsically resistant to these antibiotics. Resistant to colistin is the matter of grave concern as it is used as last resort of treatment in gram negative bacterial infections.²⁸ However prevalence of PDR bacteria was still low, giving a hope for adopting appropriate measures to check the emergence and spread of drug resistant strains. We have not tested the isolates for fosfomycin which could be a limitation in our study.

CONCLUSION

This study highlights higher prevalence of MDR (66.12%) with a considerable number of XDR bacteria (34.32%) in our set up which is alarming. It is recommended to have knowledge of own epidemiology of drug resistant bacteria in each hospital settings by continuous surveillance which will help in implementing proper hospital infection control measures and prescribing proper antibiotics. Overuse or misuse of antibiotics must be checked through antimicrobial stewardship which is the need of hour.

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