Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 08, 2023

Original research article

Clinical isolates of multidrug resistant gram negative bacilli: Antibiotic sensitivity pattern

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Abstract

According to the WHO report on antimicrobial resistance, in the South East Asia Region 16–68% of *E.coli* are resistant to Third-generation Cephalosporins (national data) and 20–95% (published data). *E.coli* resistance to fluroquinolones is 32–64% (national data) and 65–86% (published data). In invasive isolates it is around 20%. Gram negative organisms were identified as per standard protocol by Gram stain, catalase, oxidase, motility, Oxidation-Fermentation test, nitrate reduction, indole, Methyl Red, Voges–Proskauer, citrate, urease, Triple Sugar Iron agar, sugar fermentation and amino acid decarboxylation tests. Antibiotic susceptibility testing was done on Mueller Hinton agar using Kirby-Bauer disk diffusion method as per CLSI. (58) Gram negative isolates were tested against 9 groups of antibiotics. All the 150 (100%) isolates were resistant to Amoxicillin, Amoxicillin clavulinic acid, Ciprofloxacin, Cefotaxime, Ceftriaxone, Ceftazidime and Cefuroxime. 147 (98%) isolates were resistant to Aztreonam 138 (92%) to Cotrimoxazole 134 (89.3%) to Gentamicin, 114 (76%) to Piperacillin tazobactum, 100 (66.7%) to Imipenem and 99 (66%) to Amikacin. Of the 33 urine isolates tested, 17(11.3%) were resistant to Nitrofurantoin and 29 (19.3%) were resistant to Norfloxacin.

Keywords: Antibiotic Sensitivity, *E.Coli, Multidrug Resistant Gram Negative Bacilli*

Introduction

Antimicrobial resistance is a major public health problem in South East Asian countries. It is known that the infectious disease burden in India is among the highest in the world and burden of poor sanitation and malnutrition exacerbates these conditions. The resistance spectrum of pathogens varies in different regions. Therefore local resistance patterns have to be known for appropriate antimicrobial use [1].

According to the WHO report on antimicrobial resistance, November 2014, in the South East Asia Region 16–68% of *E.coli* are resistant to Third-generation Cephalosporins (national data) and 20–95% (published data). *E.coli* resistance to fluroquinolones is 32–64% (national data) and 65–86% (published data). In invasive isolates it is around 20%.

Resistance of *Klebsiella* to Third-generation Cephalosporins is 33–80% (national data) and 5–56% (published data). *Klebsiella* resistance to Carbapenam is 0–8% (national data) and 0–39.4% (published data). In invasive isolates it is 37–40%. 0–4.9% [2].

Neisseria gonorrhoea were resistant to Third-generation cephalosporins as per GASP data. Methicillin resistant *Staphylococcus aureus* is found to be 10–26% (national data) and 46% (published data).

Fluroquinolones resistance in non-typhoidal *Salmonella* was 0.2–4%. 0–82% *Shigella* were resistant to fluroquinolones as per published data. 48% *Streptococcus pneumoniae* were resistant to penicillin as per reported national data.

In a study by Manchanda *et al*. conducted in a tertiary care centre in Delhi, 81 of 383 GNB isolates were found to be MDR (21%). Of these 81 MDR isolates 36 were found to be XDR (44.4% of MDR, and 9.3% of total GNB isolates) [3].

Dewan *et al*. reported from a study in an ICU in a tertiary care hospital in North India, of the 195 ESBL producers, XDR organisms were most frequent, followed by MDR and PDR organisms - 14 (5.6%), 113 (45.2%) and 68 (27.2%) respectively.

The infections which are caused by multidrug-resistant gram negative bacilli that produce various β lactamase enzymes have been reported with an increasing frequency. In a study by Loveena Oberoi *et al*., the prevalence of various β lactamases in the Gram negative bacteria, which included the Enterobactericeae and the nonfermenters was 70.69%, which was alarmingly high [4].

Inappropriate use (overuse, underuse and misuse) in human health, veterinary health and the agriculture sector, inadequate surveillance for magnitude and trend of AMR, and usage of antibiotics, quality and

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 08, 2023

access to drugs, lack of awareness among policy makers, practitioners, patients, pharmacists and the public in general about AMR, and lack of standard treatment guidelines for most diseases are some of the contributory factors.

After the adoption of the Jaipur Declaration in 2011, India has taken several steps to combat AMR. A National task force was set up in August 2010 to review and develop a national antibiotic policy. The National Antibiotic Policy & infection control was formulated in 2011. The National Centre for Disease Control, Delhi is the nodal institution for implementation of the national programme on containment of AMR.

A national network on surveillance of AMR and monitoring the use of antibiotics has been established. NCDC, Delhi is in the process of finalizing the National Treatment Guidelines for infectious diseases [5].

Most of the diagnostic laboratories are currently carrying out antimicrobial resistance testing but there is no proper system for quality check and national data collection. Under the national programme for containment of AMR, NCDC being the nodal reference laboratory has initiated setting up a network of 30 laboratories for AMR surveillance. This programme also has a mechanism for data collection and analyses to get the exact burden of AMR [6].

ICMR has undertaken operational research projects to ascertain the impact of AMR on public health and to improve rational use of antibiotics.

Antimicrobial resistance is the best example for ice berg phenomenon of disease with superbugs, the visible manifestations of our prolonged failure to preserve antibiotics. Paucity of data at national level makes it difficult to understand the magnitude of the problem and various factors responsible for emergence of antimicrobial resistance.

Methodology

Inclusion criteria

Multidrug resistant Gram negative clinical isolates

Exclusion criteria

1. Non multidrug resistant Gram negative organisms
2. All Gram positive organisms
3. All Gram negative cocci
4. All organisms showing inherent resistance to Colistin such as *Proteus species*, *Vibrio species*, *Burkholderia species.*

Gram negative organisms were identified as per standard protocol by Gram stain, catalase, oxidase, motility, Oxidation-Fermentation test, nitrate reduction, indole, Methyl Red, Voges–Proskauer, citrate, urease, Triple Sugar Iron agar, sugar fermentation and amino acid decarboxylation tests.

Antibiotic susceptibility testing was done on Mueller Hinton agar using Kirby-Bauer disk diffusion method as per CLSI. Gram negative isolates were tested against 9 groups of antibiotics.

Results

Table 1: Resistance pattern of the isolates

Antibiotics	Resistance	
	Number	Percentage
Amoxicillin	150	100%
Amoxicillin clavulinic acid	150	100%
Ceftriaxone	150	100%
Cefotaxime	150	100%
Ceftazidime	150	100%
Cefuroxime	150	100%
Ciprofloxacin	150	100%
Gentamicin	134	89.3%
Cotrimoxazole	138	92%
Imipenem	100	66.7%
Amikacin	99	66%
Aztreonam	147	98%
Piperacillin tazobactum	114	76%
Norfloxacin	29/33*	19.3%
Nitrofurantoin	17/33*	11.3%

*urine isolates were only 33.

All the 150 (100%) isolates were resistant to Amoxicillin, Amoxicillin clavulinic acid, Ciprofloxacin, Cefotaxime, Ceftriaxone, Ceftazidime and Cefuroxime.

147 (98%) isolates were resistant to Aztreonam 138 (92%) to Cotrimoxazole 134 (89.3%) to Gentamicin,

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114 (76%) to Piperacillin tazobactum, 100 (66.7%) to Imipenem and 99 (66%) to Amikacin.
Of the 33 urine isolates tested, 17(11.3%) were resistant to Nitrofurantoin and 29 (19.3%) were resistant to Norfloxacin.

Table 2: Resistance pattern organism wise

Antibiotics	***Acinetobacter* Spp.**		***Citrobacter* spp.**		***E.coli***		***Enterobacter* spp.**		***Klebsiella* spp.**		***Pseudomonas* spp.**	
Amox	23	100 %	10	100 %	54	100 %	6	100 %	39	100 %	18	100 %
AC	23	100 %	10	100 %	54	100 %	6	100 %	39	100 %	18	100 %
CI	23	100 %	10	100 %	54	100 %	6	100 %	39	100 %	18	100 %
CE	23	100.0 %	10	100 %	54	100 %	6	100 %	39	100 %	18	100 %
CA	23	100.0 %	10	100 %	54	100 %	6	100 %	39	100 %	18	100 %
CF	23	100.0 %	10	100 %	54	100 %	6	100 %	39	100 %	18	100 %
G	21	91.3 %	10	100 %	43	79.6 %	5	83.3 %	39	100 %	16	88.9 %
CO	23	100 %	9	90 %	49	90.7 %	5	83.3 %	34	87.2 %	18	100 %
I	17	73.9 %	8	80 %	35	64.8 %	4	66.7 %	22	56.4 %	14	77.8 %
AK	20	87 %	6	60 %	27	50%	2	33.3 %	28	71.8 %	16	88.9 %
AO	23	100 %	10	100 %	51	94.4 %	6	100 %	39	100 %	18	100 %
CU	23	100 %	10	100 %	54	100 %	6	100 %	39	100 %	18	100 %
NF	0	.0	1	10 %	10	18.5 %	1	16.7 %	5	12.8 %	0	.0
NX	0	.0	4	40 %	19	35.2 %	1	16.7 %	5	12.8 %	0	.0
PT	18	78.3 %	7	70 %	41	75.9 %	2	33.3 %	31	79.5 %	15	83.3 %

Acinetobacter spp. showed resistance to beta lactams, cephalosporins, ciprofloxacin, co trimoxazole and aztreonam, moderate sensitivity to gentamicin, amikacin, imipenem and piperacillin tazobactum.
Citrobacter spp. showed resistance to beta lactams, cephalosporins, gentamicin, ciprofloxacin and aztreonam, moderate sensitivity to cotrimoxazole, imipenem, amikacin, piperacillin tazobactum, nitrofurantoin and norfloxacin.
E.coli showed resistance to beta lactams, cephalosporins, gentamicin and ciprofloxacin, moderate sensitivity to cotrimoxazole, imipenem, amikacin, aztreonam, piperacillin tazobactum, nitrofurantoin and norfloxacin.
Enterobacter spp. showed resistance to beta lactams, cephalosporins, ciprofloxacin, aztreonam, moderate sensitivity to gentamicin, cotrimoxazole, imipenem, amikacin, aztreonam, piperacillin tazobactum, nitrofurantoin and norfloxacin.
Klebsiella spp. showed resistance to beta lactams, cephalosporins, ciprofloxacin, gentamicin, and aztreonam, moderate sensitivity to cotrimoxazole, imipenem, amikacin, piperacillin tazobactum, nitrofurantoin and norfloxacin.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 08, 2023

Pseudomonas spp. showed resistance to beta lactams, cephalosporins, ciprofloxacin, cotrimoxazole, moderate sensitivity to gentamicin, imipenem, amikacin and piperacillin tazobactum.

Table 3: Beta lactamase producers

		Number	Percentage
ESBL	NP	117	78.0
	P	33	22.0
MBL	NP	96	64.0
	P	54	36
AMP C	NP	131	87.3
	P	19	12.7

ESBL- Extended spectrum beta lactamase, MBL- metallo beta lactamase, NP- nonproducer, P- producer.

Of the 150 isolates, 33 (22%) were ESBL producers, 54 (36%) MBL producers, 19 (12.7%) were Amp C producers.

Table 4: Beta lactamase producers organism wise

		***Acinetobacter* Spp.**		***Citrobacter* spp.**		***E.coli***		***Enterobacter* spp.**		***Klebsiella* spp.**		***Pseudomonas* spp.**	
ESBL	NP	21	91.3	9	90.0	30	55.6	5	83.3	34	87.2	18	100.0
	P	2	8.7	1	10.0	24	44.4	1	16.7	5	12.8	0	.0
MBL	NP	14	60.9	8	80.0	41	75.9	6	100.0	20	51.3	7	38.9
	P	9	39.1	2	20.0	13	24.1	0	.0	19	48.7	11	61.1
AMP C	NP	20	87.0	8	80.0	44	81.5	5	83.3	37	94.9	17	94.4
	P	3	13.0	2	20.0	10	18.5	1	16.7	2	5.1	1	5.6

ESBL- Extended spectrum beta lactamase, MBL- metallo beta lactamase, NP- nonproducer, P- producer.

24 (44.4%) *E.coli*, 5 (12.8%) *Klebsiella*, 2 (8.7%) *Acinetobacter*, 1(16.7%) *Enterobacter*, 1 (10%) *Citrobacter* were ESBL producers.
19 (48.7%) *Klebsiella*, 13 (24.1%) *E.coli*, 11 (61.1%) *Pseudomonas*, 9 (39.1%) *Acinetobacter* and 2 (20%) *Citrobacter* were MBL producers.
10 (18.5%) *E.coli*, 3 (13%) *Acinetobacter*, 2 (20%) *Citrobacter*, 1 (16.7%) *Enterobacter*, 2 (5.1%) *Klebsiella* and 1 (5.6%) *Pseudomonas* were Amp C producers.

Discussion

Table 5: Antibiotic resistance pattern of isolates in various studies

Study	**Year**	**ß lactams & Cephalosporins**	**Amg.**	**Quinolones**	**Carbapenems**	**Monobactam**
Behera *et al.*[7]	2007	45%	45 %	45%	45 %	45 %
Zubair *et al.* [8]	2010	79.4%	55.5 %	57.1%	7.1%	57.1%
Vinod kumar *et al.* [9]	2011	79.5%	74.6 %	84.3%	53%	53%
Mezzatesta *et al.* [10]	2013	100%	100%	100%	100%	100%
Rajput & Naik [11]	2013	63%	Not tested	Not tested	56%	63%
Samant *et al.* [12]	2013	67.3%	63.3 %	73.3%	48.5%	Not tested
Present study	2014	100 %	89.3 %	100%	66.7%	98%

(amg- aminoglycosides)

In the present study, 100 % resistance was noted to ß lactams, cephalosporins and quinolones which

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 08, 2023

corresponded with Mezzatesta *et al*.
The MDROs in the present study also showed a high resistance of 98% to monobactams which corresponded with Mezzatesta *et al*. but was higher than other studies.
Samant *et al*.and Vinod kumar *et al*.noted a high resistance of 63.3% and 74.6% respectively to aminoglycosides. Present study showed 89.3% resistance to aminoglycosides. A higher resistance of 66.7% to carbapenems was seen in present study as compared to studies by Rajput & Naik and Vinod kumar *et al*.
In the present study, few multidrug resistant strains showed coresistance to the fluoroquinolones and the aminoglycosides, but they were moderately susceptible to imipenem and the piperacillin- tazobactam combination, which was in concordance with the findings of other studies.
Various factors like patient demographics, length of hospital stay, presence of invasive devices, co-morbid conditions, admission to ICU, source of sample, prior use of antibiotics or inappropriate dosing, different mechanisms of drug resistance in the organisms and their transfer could be responsible for such a pattern of multidrug resistance.

Table 6: Comparison of beta lactamase producers with other studies

Study	Year	Place	ESBL	MBL	Amp C
Nagdeo *et al*. [13]	2009	Bhopal	39.3%	7.44%	9.29%
Loveena *et al*.[14]	2013	Amritsar	35.16%	10.98%	5.4%
Bareja *et al*.[15]	2013	Harayana	30.83%	-	15.35%
Altun *et al*.[16]	2013	Turkey	28%	43.5%	75.64%
Present study	2014	MMCRI,Mysore	22%	36%	12.7%

Present study showed higher MBL producers -36% which correlated with the study of Altun *et al*. ESBL levels in present study also correlated with other studies. It has been proved that the prevalence of the ESBLs among the clinical isolates varies from country to country and institution to institution within the same country.
Low level of Amp C producers from present study correlated with other studies. The low prevalence of the AmpC producers in our study could be due to the differences in the geographical distribution, which may have produced variations in the prevalence of the β-lactamases which may have been present in the different organisms, which may have given rise to the varied resistance patterns.
The coexistence of different classes of β-lactamases in a single bacterial isolate may pose diagnostic and treatment challenges. The AmpC producing organisms can act as a hidden reservoir for the ESBLs. Also, the high-level expression of the AmpC β-lactamases may mask the recognition of the ESBLs and it may result in a fatal and an inappropriate antimicrobial therapy.
The increase in the prevalence of the AmpC, MBL and the ESBL producing isolates may be indicative of the ominous trend of more and more isolates acquiring the resistance mechanisms, thus rendering the antimicrobial armamarium ineffective.

Conclusion

- All the 150 (100%) isolates were resistant to Amoxicillin, Amoxicillin clavulinic acid, Ciprofloxacin, Cefotaxime, Ceftriaxone, Ceftazidime and Cefuroxime.
- 147 (98%) isolates were resistant to Aztreonam, 138 (92%) to Cotrimoxazole, 134 (89.3%) to Gentamicin, 114 (76%) to Piperacillin tazobactum, 100 (66.7%) to Imipenem and 99 (66%) to Amikacin.
- Of the 33 urine isolates tested, 17(11.3%) were resistant to Nitrofurantoin and 29 (19.3%) were resistant to Norfloxacin.

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