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DRUG RESISTANCE PROFILE OF CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL FROM HARYANA

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ABSTRACT Antimicrobial drug resistance is a major challenge today especially with multidrug resistant pathogens in a tertiary care hospital. The drug resistant pattern must be reviewed and assessed periodically to formulate the hospital policies and understanding the extent of drug resistance. A retrospective study was conducted on all the clinical isolates reported in our hospital between 05 November 2020 to 08 July 2021. Altogether 179 isolates were reported. *Escherichia coli* was the commonest, followed by *Klebsiella pneumoniae, Staphylococcus aureus* and *Acinetobacter spp.* Amongst Gram negative Enterobacterales, carbapenem resistance was 23.1%, cephalosporin resistance 83.7%, fluoroquinolone resistance 89.4%, aminoglycoside resistance more than 28.8% and tigecycline resistance 5.9%. Amongst the Gram negative Nonfermenters, *Acinetobacter spp* had 100% carbapenem, cephalosporin, and fluoroquinolone resistance whereas tigecycline was resistant in 51.8%. Amongst the Gram positive cocci, Methicillin resistant *Staphylococcus aureus* was 50% however, none of the Gram positive cocci including *Enterococcus faecalis* were vancomycin resistant. The pathogens of significant concern were the multidrug resistant *Acinetobacter spp.* and *Klebsiella pneumoniae* especially with higher rates of carbapenem resistance besides Methicillin resistant *Staphylococcus aureus.*

KEYWORDS : Dug resistance, Carbapenem resistance, Cephalosporin resistance, MRSA

INTRODUCTION

Antimicrobial drug resistance is a persistant challenge for the healthcare. Significant efforts are going on presently to curtail the spread of drug resistance but it continues to be a challenge despite hospital infection control policies and antimicrobial stewardship programs.

The multidrug resistant (MDR) Gram negative bacteria are the commonest pathogens that persist in the hospital environment.[1] Studies have observed that 60 to 80% of the Gram negative bacteria are extended spectrum beta lactamase (ESBL) producers.[1] The prevalence of carbapenem resistance is also on the rise with the emergence of NDM producing *Klebsiella pneumoniae*.[2] In a study from multicenter ICUs *Klebsiella spp* resistant to third generation cephalosporins and carbapenems or carbapenem-resistant *Acinetobacter spp* was independently associated with a higher risk of death and infection with another microorganism.[3] Amongst Gram positive cocci the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) could range upto 60%,[4] whereas the Vancomycin Resistant Enterococcus ranges from 1 to 9%.[5]

The drug resistance is a major concern when the organisms are reported as Multidrug resistant. The assessment of drug resistance in a tertiary care hospital is a need of the hour so that the treating doctor and hospital management is aware of the drug resistance trends, and hospital policies are formulated with that data to curb the spread of MDR organisms (MDROs). Therefore, this study was undertaken to assess the pattern of drug resistance in the organisms isolated from the various clinical specimens over a period of eight months in our hospital.

MATERIAL & METHODS

The study was conducted at Adesh Medical College and Hospital, Kurukshetra, Haryana with due approval by the institutional ethics and research committee (IERC). All the nonduplicate clinical isolates reported by the bacteriology lab during eight months period, from 05 November 2020 to 08 July 2021 were included in this retrospective study where the data was collected from the lab records.

The conventional bacterial culture and identification techniques were used for the various clinical specimens. For drug susceptibility testing and interpretation, Clinical & Lab Standards Institute (CLSI) approved methods and breakpoints were used. Any specimen which was received more than once from the same patient was excluded from the analysis.

RESULTS

The study included 179 clinical isolates which were reported during the study period from the various clinical specimens. The distribution of various species (Table 1) and nature of clinical specimens (Table 2) are shown below. The commonest reported organism was *Escherichia coli* (34.6%) followed by *Klebsiella pneumoniae* (22.3%) and *Staphylococcus aureus* (16.8%). The commonest specimen from which a bacterium was isolated was urine (41.3%) followed by pus (25.1%) and tracheal secretions (8.9%).

Table 1: Distribution of organisms

Organism	Number of isolates (%)
Escherichia coli	62 (34.6%)
Klebsiella pneumoniae	40 (22.3%)
Staphylococcus aureus	30 (16.8%)
Acinetobacter spp.	27 (15.1%)
Pseudomonas aeruginosa	14 (7.8%)
Enterococcus faecalis	4 (2.2%)
Proteus vulgaris	2 (1.1%)
Total	**179**

Table 2: Distribution of clinical specimens

Specimen	Number of specimens (%)
Urine	74 (41.3%)
Pus	45 (25.1%)
Tracheal secretions	16 (8.9%)
Sputum	9 (5%)
Excised tissue	9 (5%)
Bronchoalveolar lavage fluid	8 (4.5%)
Wound swab	7 (3.9%)
Body fluid	5 (2.8%)
Vaginal swab	4 (2.2%)
Dialysis catheter tip	1 (0.6%)
Intercostal drain tip	1 (0.6%)
Total	**179**

Altogether 104 isolates of Gram negative bacteria belonging to the family Enterobacterales were reported during the study period. Of these, *Escherichia coli* was reported in 62, *Klebsiella pneumoniae* in 40 and *Proteus vulgaris* in 2 specimens. The distribution of drug resistance is presented in Table 3 below.

Table 3: Enterobacterales: Distribution of drug resistance

Antibiotic	*Escherichia coli* (%) (n=62)	*Klebsiella pneumoniae* (%) (n=40)	*Proteus vulgaris* (n=2)	Total resistant (%)
Imipenem	2 (3.2%)	21 (52.5%)	1	23.1%
Meropenem	2 (3.2%)	21 (52.5%)	1	23.1%
Piperacillin/ Tazobactam	11 (17.7%)	30 (75%)	1	40.4%
Ceftazidime/ Clavulanate	11 (17.7%)	30 (75%)	1	40.4%
Cefepime	47 (75.8%)	34 (85%)	2	79.8%
Aztreonam	37 (59.7%)	32 (80%)	2	68.3%
Ceftazidime	51 (82.3%)	34 (85%)	2	83.7%
Cefotaxime	51 (82.3%)	34 (85%)	2	83.7%
Ceftriaxone	51 (82.3%)	34 (85%)	2	83.7%
Cefuroxime	56 (90.3%)	37 (92.5%)	2	91.3%
Amoxiclav	50 (80.6%)	36 (90%)	2	84.6%
Gentamicin	34 (54.8%)	27 (67.5%)	2	60.6%
Amikacin	6 (9.7%)	24 (60%)	0	28.8%
Netilmicin	14 (22.6%)	26 (65%)	2	40.4%
Tobramycin	27 (43.5%)	23 (57.5%)	2	50.0%
Norfloxacin	44 (71%)	6 (15%)	0	48.1%
Ciprofloxacin	55 (88.7%)	36 (90%)	2	89.4%
Ofloxacin	55 (88.7%)	36 (90%)	2	89.4%
Levofloxacin	55 (88.7%)	36 (90%)	2	89.4%
Cotrimoxazole	50 (80.6%)	35 (87.5%)	2	83.7%
Tetracycline	55 (88.7%)	38 (95%)	2	91.3%
Colistin	0	0	NT	0.0%
Tigecycline	1 (1.6%)	5 (12.5%)	NT	5.9%
Nitrofurantoin	5 (8.8%)	4 (7%)	NT	15.8%
Fosfomycin	0	0	0	0.0%

NT: Not tested (as per CLSI guidelines)

The highest proportion of drug resistance amongst Enterobacterales was contributed by *Klebsiella pneumoniae.* Carbapenem resistance was reported in 52.5% whereas cephalosporin resistance was observed in more than 85% of *Klebsiella pneumoniae.* Fluoroquinolone resistance was observed in 90% of the *Klebsiella pneumoniae,* while aminoglycoside resistance varied between 57.5% (tobramycin) to 67.5% (gentamicin). None of the *Klebsiella pneumoniae* were reported resistant to colistin however tigecycline resistance was reported in 12.5%. None of the isolates were reported resistant to fosfomycin however, nitrofurantoin resistance was observed in 7%.

Amongst *Escherichia coli*, the drug resistance was lower compared to *Klebsiella pneumoniae.* Carbapenem resistance was observed in 3.2% isolates. The cephalosporin resistance and fluroquinolone resistance were observed in more than 82.3% and 88.7% respectively, which was comparable to *Klebsiella pneumoniae*. The resistance to gentamicin was 54.8% however, amikacin resistance was significantly lower at 9.7%. Colistin resistance was not observed however tigecycline resistance was observed in 1.6%. None of the urinary isolates were reported resistant to fosfomycin however 8.8% were reported as resistant to nitrofurantoin.

Proteus vulgaris though reported in only two specimens, was observed to have a resistant pattern which included carbapenem resistance, cephalosporin resistance, fluoroquinolone resistance and aminoglycoside resistance.

Overall, amongst the Enterobacterales, carbapenem resistance was observed to be 23.1% and cephalosporin resistance was more than 83.7%. The fluoroquinolone resistance was 89.4% whereas aminoglycoside resistance varied between 28.8% (amikacin) and 60.6% (gentamicin). Tigecycline resistance was also observed in 5.9% of isolates.

The distribution of drug resistance among Gram negative Nonfermenters is shown in Table 4 below. Of the 41 isolates, *Acinetobacter spp.* was reported in 27 whereas *Pseudomonas aeruginosa* was reported in 14 clinical specimens.

Table 4: Gram Negative Nonfermenters: Distribution of drug resistance

Antibiotic	*Acinetobacter species* (%) (n=27)	*Pseudomonas aeruginosa* (%) (n=14)	Total resistant (%)
Imipenem	27 (100%)	5 (35.7%)	78.0%
Meropenem	27 (100%)	5 (35.7%)	78.0%
Piperacillin/ Tazobactam	27 (100%)	6 (42.9%)	80.5%
Ceftazidime/ Clavulanate	27 (100%)	6 (42.9%)	80.5%
Cefuroxime	27 (100%)	14 (100%)	100.0%
Cefotaxime	27 (100%)	NT	-*
Ceftriaxone	27 (100%)	NT	-*
Ceftazidime	27 (100%)	8 (57.1%)	85.4%
Cefepime	27 (100%)	6 (42.9%)	80.5%
Aztreonam	27 (100%)	4 (28.6%)	75.6%
Gentamicin	26 (96.3%)	9 (64.3%)	85.4%
Amikacin	25 (92.6%)	6 (42.9%)	75.6%
Netilmicin	26 (96.3%)	8 (57.1%)	82.9%
Tobramycin	23 (85.2%)	5 (35.7%)	68.3%
Norfloxacin	4 (14.8%)	2 (14.3%)	14.6%
Ciprofloxacin	27 (100%)	6 (42.9%)	80.5%
Levofloxacin	27 (100%)	6 (42.9%)	80.5%
Tetracycline	27 (100%)	NT	-*
Colistin	0	0	0.0%
Tigecycline	14 (51.8%)	NT	-*
Fosfomycin	2 (50%)	0	20.0%
Ampicillin/ sulbactam	26 (96.3%)	NT	-*

NT: Not tested (as per CLSI guidelines);

* Total resistance not specified as one of the listed organisms was not tested for the drug as per CLSI guidelines

Acinetobacter spp. was observed to be highly resistant. Carbapenem resistance and cephalosporin resistance was observed in all the isolates. Almost 90% or more drug resistance was observed with aminoglycosides and fluoroquinolones. Tigecycline and tetracycline resistance was observed in 51.8% and 100% respectively. The urinary isolates were resistant to fosfomycin in 50% whereas none were resistant to colistin.

Pseudomonas aeruginosa was observed to have a lower resistance compared to *Acinetobacter spp.* Carbapenem resistance was observed in 35.7% whereas cephalosporin resistance was observed in more than 57.1%. Aminoglycoside resistance was observed to be between 35.7% (tobramycin) and 64.3% (gentamicin) whereas fluoroquinolone resistance was observed to be lower ranging between 14.3% (norfloxacin) to 42.9% (levofloxacin). None of the isolates were reported as resistant to colistin.

The distribution of drug resistance among Gram positive cocci is shown in Table 5 below. Of the 34 Gram positive cocci, *Staphylococcus aureus* was reported in 30 whereas *Enterococcus faecalis* was reported in 4 clinical specimens.

Table 5: Gram Positive Cocci: Distribution of drug resistance

Antibiotic	*Staphylococcus aureus* (%) (n=30)	*Enterococcus faecalis* (n=4)	Overall resistant (%)
Ampicillin	29 (96.7%)	1	88.2%
Amoxiclav	17 (56.6%)	NT	-*
Cefazolin	16 (53.3%)	NT	-*
Cefuroxime	15 (50%)	NT	-*
Cefotaxime	15 (50%)	NT	-*
Cefoxitin	15 (50%)	NT	-*
Gentamicin	10 (33.3%)	2	35.3%
Netilmicin	5 (16.7%)	NT	-*
Ciprofloxacin	27 (90%)	3	88.2%
Levofloxacin	27 (90%)	3	88.2%
Cotrimoxazole	10 (33.3%)	4	41.2%
Tetracycline	8 (26.7%)	3	32.4%
Erythromycin	21 (70%)	1	64.7%
Azithromycin	20 (66.7%)	0	58.8%
Clindamycin	11 (36.7%)	1	35.3%

Teicoplanin	0	0	0.0%
Linezolid	0	0	0.0%
Vancomycin	0	0	0.0%
Nitrofurantoin	0	1	14.3%

NT: Not tested (as per CLSI guidelines);

* Total resistance not specified as one of the listed organisms was not tested for the drug as per CLSI guidelines

Of the *Staphylococcus aureus* isolated from clinical specimens, 50% were reported as Methicillin resistant. Beta lactam resistance was observed to be 50% to 96%, fluoroquinolone resistance was 90% whereas aminoglycoside resistance was between 16.7% (netilmicin) and 33.3% (gentamicin). Erythromycin and azithromycin were resistant in 70% and 66.7% respectively whereas clindamycin was resistant in 36.7%. None of the isolates were reported as resistant to teicoplanin, vancomycin and linezolid.

Enterococcus faecalis was isolated in very few clinical specimens (n=4) however, it was observed to be resistant to fluoroquinolones and aminoglycosides. None of the four isolates were resistant to vancomycin.

DISCUSSION

Drug resistance is an important concern in the tertiary care hospitals today which is significantly impacting the patient treatment outcomes. The hospitals have to constantly evolve policies customized to individual institutional needs. However, the policies need to be data driven therefore this study was undertaken in our hospital to assess the extent of drug resistance reported in various clinical isolates.

Throughout the study period, 179 clinical isolates were reported. The commonest reported organism amongst the Enterobacterales Gram negative bacteria (n=104) was *Escherichia coli* (34.6%), amongst Nonfermenting Gram negative bacteria (n=41) commonest was *Acinetobacter spp.* (15.1%), whereas amongst the Gram positive cocci (n=34) commonest was *Staphylococcus aureus* (16.8%). A bacterial growth was most commonly reported from the urine specimen (41.3%) which is also the likely explanation that *Escherichia coli* was the commonest reported pathogen in our study since it is the commonest reported uropathogen as well.[6]

The carbapenem resistance and third generation cephalosporin resistance was observed in 23.1% and 83.7% of Enterobacterales. Although the extent of cephalosporin resistance was almost comparable across the various Gram negative Enterobacterales but the carbapenem resistance was significantly contributed by *Klebsiella pneumoniae* (21 of 24 isolates). The aminoglycoside resistance was also observed to be higher for netilmicin and amikacin. These observed drug resistance patterns are almost comparable to another study from India with reported carbapenem resistance as 10-50%, cephalosporin resistance 68-90%, fluoroquinolone resistance 74-90%, and aminoglycoside resistance 48-76%.[1] Tigecycline resistance in our study was 5.9% with higher contribution by the carbapenem resistant *Klebsiella pneumoniae* (5 of 6 isolates). This finding was almost similar to another study which reported 8.3% resistance amongst *Escherichia coli* and *Klebsiella pneumoniae* with higher proportion contributed by carbapenem resistant Enterobacteriacae.[7]

Amongst the Gram negative Nonfermenters, *Acinetobacter spp.* was observed to have a 100% carbapenem and cephalosporin resistance besides fluoroquinolones. Our reported rates are higher compared to another study which observed 83% carbapenem resistance, 90% cephalosporin resistance, and 86% fluoroquinolonone resistance.[1] Our study observed comparatively lower rates of drug resistance for *Pseudomonas aeruginosa* wherein carbapenem resistance was almost 35%, cephalosporin resistance 57% and fluoroquinolone resistance 43%. This was observed to be slightly lower when compared to another similar study with reported carbapenem resistance as 56%, cephalosporin resistance 74% and fluoroquinolone resistance as 67%.[1] The variation may have been contributed by smaller number of isolates reported in our study. Tigecycline resistance was observed in almost 51.8% of the *Acinetobacter spp.* which is comparable to another study which reported 45% resistance.[8]

Amongst the Gram positive cocci, Methicillin resistant *Staphylococcus aureus* was reported in 50% of the cases. This is similar to the various other studies from India which have observed this in the range of 32-80%.[9]

The pathogens of significant concern presently in our hospital are the MDR *Acinetobacter spp.* and *Klebsiella pneumoniae* with carbapenem resistance besides Methicillin resistant *Staphylococcus aureus.* The antimicrobial stewardship program should take adequate steps through the various policies to curtail the transmission of these pathogens.

In conclusion, a high rate of carbapenem resistance was observed amongst *Acinetobacter spp.* and *Klebsiella pneumoniae* besides cephalosporin and fluroquinolone resistance. Most of these organisms were resistant to multiple antibiotics. Hospitals must assess and intervene to control such multidrug resistant pathogens in the hospital.

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