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Profile and Drug Susceptibility Pattern of Gram Negative Bacterial Isolates - A Retrospective Institutional Study

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Abstract
Background: *Antimicrobial resistance in bacteria is a growing problem though not a new phenomenon nor unexpected, especially in an environment where potent antibiotics are used very frequently. The drug resistant strains can spread rapidly in hospital settings, especially if the infection control measures are not strictly implemented and followed.*
Materials and Methods: *A retrospective descriptive study was conducted on the profile and susceptibility pattern of Gram negative bacterial isolates obtained from various clinical specimens over a period of 8 years (1999-2007). The data was collected from the laboratory records. Study period was divided into2 – Period A (1999-2004) and Period B (2005-2007). The profile and antibiotic sensitivity pattern of Gram negative bacteria from each period was compared.*
Results: *E.coli and Klebsiella species, the first and second commonest urine isolates , showed significant increase in resistance to 1st & 2nd generation Cephalosporins and quinolones (p value <0.05). Blood isolates of E.coli & Klebsiela showed significant increase in resistance to quinolones & 3rd generation Cephalosporins and a decrease in resistance to aminoglycosides. For Pseudomonas species, significant increase in resistance to Amikacin, Gentamicin and Ciprofloxacin and a decrease in resistance to Ceftazidime was documented between the two study periods. Overall, there is an increase in MDR GNB isolates from all groups of specimens.*
Conclusion: *High resistance documented to commonly used Cephalosporins, Ceftriaxone and Cefotaxime for most Gram negative bacteria isolated from different sites and an increase in the rate of isolation of multi drug resistant strains.*
Keywords: *Gram negative bacteria/ Drug susceptibility/ Drug resistance.*

Background

Antibiotic resistance is an emerging problem crippling infectious disease management around the globe. Up to 70% of nosocomial infections, are caused by organisms that are resistant to at least one antimicrobial agent previously known to be effective[(1)]. Over the past two decades, microbial resistance to antibiotics is on increase for several species of Gram Negative bacteria[(2) (3)], which mainly include extended-spectrum Beta-lactamases (ESBLs) in Klebsiella pneumoniae, Escherichia coli, and Proteus mirabilis and multidrug-resistance in Pseudomonas aeruginosa, Acinetobacter, and Stenotrophomonas maltophilia [(3)]. A regional difference in the prevalence of resistance is reported in Indian studies. A study

from PGI Chandigarh, reports the prevalence of ESBL producers among Gram negative uropathogens as 36.5 %,[4] whereas the study from AFMC Pune report this as 22%. [5] Locally generated data, regarding the profile of bacterial isolates and their susceptibility patterns, is therefore of paramount importance for formulating and modifying the hospital infection control protocols and this would also help the clinician in selecting empirical antibiotic therapy. For the individual patient, especially one who is critically ill, this could make a difference, often one between life and death.

Objectives

1) To study the profile and drug resistance pattern of Gram Negative Bacterial isolates obtained from various clinical specimens of patients treated at a tertiary care centre in Central Kerala.
2) To observe the evolution of drug resistance over the years, by dividing the study period of 8 years into two halves (4 years each) and compare the profile and antibiotic resistance patterns of GNB isolates in both periods.

Materials and Methods

A Retrospective Institutional Descriptive study. The data includes the ABST pattern as per "Disc diffusion method", of all Gram Negative Bacterial isolates obtained from various clinical specimens over a period of 8 years (1999 to 2007). The study period was grouped into 2 - Period A & Period B. Period A denotes 1999-2004 and Period B denotes 2005-2007. Prevalence of MDR GNB isolates and resistance rates of each isolate to individual antibiotics were compared during both periods.

Results

Total of 3482 Gram negative bacterial isolates were studied and the isolates vs specimens distribution is as below.

Table: 1

Specimen	Number of GNB isolates
Urine	2293
Aspirates & swabs	837
Blood	352
Total	**3482**

Table: 2 Profile of GNB isolates from urine

Bacterial Isolate	Period A		Period B	
	Total No. of isolates	Percentage	Total No. of isolates	Percentage
E.coli	666	52.9	709	68.6
Klebsiella	375	29.8	256	24.8
Coliforms	130	10.3	17	1.6
Pseudomonas	32	2.5	36	3.5
Proteus	32	2.5	10	1
Acinetobacter	18	1.4	4	0.4
Alk.faecalis	2	0.2	0	0
S.paratyphi	2	0.2	0	0
S.typhi	2	0.2	0	0
Enterobacter	1	0.1	0	0
Flavobacteria	0	0	1	0.1
TOTAL	**1260**	**100%**	**1033**	**100%**

E.coli was the most common isolate obtained from urine, followed by Klebsiella spp.

Table 3 Profile of GNB isolates from aspirates and swabs

	Period A		Period B	
	Total No. of isolates	**Percentage**	**Total No. of isolates**	**Percentage**
Pseudomonas	133	35	145	31.7
Klebsiella	104	27.4	141	30.9
E.coli	68	17.9	108	23.6
Coliforms	25	6.6	6	1.3
Proteus	35	9.2	31	6.8
Acinetobacter	15	3.9	20	4.4
S.typhi	0	0	1	0.2
Enterobacter	0	0	2	0.4
Flavobacteria	0	0	1	0.2
Providentia	0	0	1	0.2
H.influenza	0	0	1	0.2
TOTAL	**380**	**100%**	**457**	**100%**

Pseudomonas aeruginosa was the most frequently isolate from various aspirates & swabs, closely followed by Klebsiella spp and this pattern was preserved in both periods.

Table: 4 Profile of GNB isolates from Blood

	Period A		Period B	
	Total isolates	**%**	**Total isolates**	**%**
Acinetobacter	**54**	27	**34**	22.5
Klebsiella	**48**	24	**69**	45.7
S.typhi	25	12.5	2	1.3
Pseudomonas	**23**	11.5	**16**	10.6
Coliforms	21	10.5	9	6
E.coli	16	8	19	12.6
S.paratyphi	6	3	0	0
Proteus	2	1	1	0.7
Flavobacteria	2	1	1	0.7
Alk.faecalis	1	0.5	0	0
Enterobacter	1	0.5	0	0
H.influenza	1	0.5	0	0
TOTAL	**200**	100	**151**	100

Acinetobacter and Klebsiella were the common blood isolates in both periods. Klebsiella showed an increase in prevalence and Acinetobacter a decrease, in Period B compared to Period A.

Comparison of Resistance to Individual Antibiotics by E.Coli during Period A & Period B

Table: 4 Urine isolates

	Period A			Period B			
ANTIBIOTICS	**No. of isolates tested**	**No. of resistant E.coli**	**Resistance %**	**No. of isolates tested**	**No. of resistant E.coli**	**Resistance %**	**P value**
Ampicillin	589	547	92.9	614	562	91.5	0.22
Cephelexin	493	334	**67.7**	560	408	**72.9**	**0.04**
Gentamicin	415	177	42.7	551	237	46.6	0.12
Amikacin	167	21	12.6	304	48	15.8	0.21
Ciprofloxacin	163	105	**64.4**	269	223	**82.9**	**0**
Cefotaxime	36	19	**52.8**	137	105	**76.6**	**0.005**
Ceftriaxone	127	67	**52.8**	133	103	**77.4**	**0**
Cotrimoxazole	505	353	69.9	593	404	68.1	0.285
Nalidixic acid	536	435	81.2	265	222	83.8	0.2
Nitrofurantoin	627	158	25.2	289	76	26.3	0.39
Norfloxacin	574	359	**62.5**	560	419	**74.8**	**0**

A significant increase in invitro resistance was seen to 1st & 3rd generation Cephalosporins & quinolones.

Table: 5 Isolates from Aspirates & swabs

	Period A			Period B			
ANTIBIOTICS	**No. of isolates tested**	**No. of resistant E.coli**	**Resistance %**	**No. of isolates tested**	**No. of resistant E.coli**	**Resistance %**	**P value**
Ampicillin	63	59	93.7	89	81	91	0.39
Cephelexin	53	40	75.5	96	79	82.3	0.216
Gentamicin	55	30	54.5	87	51	58.7	0.38
Amikacin	23	6	26.1	42	6	14.3	0.19
Ciprofloxacin	55	30	**54.5**	88	67	**76.1**	**0.006**
Cefotaxime	24	8	53.3	43	31	62.9	0.222
Ceftriaxone	30	16	**33.3**	61	39	**72.1**	**0.002**
Cotrimoxazole	57	37	64.9	79	53	67.1	0.466

E.coli showed a significant increase in resistance to Ciprofloxacin & Ceftriaxone. Except for Amikacin, there was an increase in resistance to all other antibiotics tested.

Table: 6 Blood isolates

	Period A			Period B			
ANTIBIOTICS	**No. of isolates tested**	**No. of resistant E.coli**	**Resistance %**	**No. of isolates tested**	**No. of resistant E.coli**	**Resistance %**	**P value**
Ampicillin	14	13	92.9	17	15	88.2	0.57
Cephelexin	12	9	75	18	15	83.3	0.45
Gentamicin	13	6	46.2	16	11	68.8	0.19
Amikacin	4	3	75	4	2	50	0.5
Ciprofloxacin	10	4	40	16	10	62.5	0.27
Cefotaxime	12	4	33.3	8	5	62.5	0.2
Ceftriaxone	9	5	55.6	8	8	100	0.052
Cotrimoxazole	12	5	41.7	18	11	61.1	0.25

E.coli isolated from blood did not show a significant change in resistance during both groups of years to any of the antibiotic tested**.**

Comparison of Resistance to Individual Antibiotics by Klebsiella during Period A & Period B

Table: 7 Urine isolates

	Period A			Period B			P value
ANTIBIOTICS	**No. of isolates tested**	**No. of resistant Klebsiella**	**Resistance %**	**No. of isolates tested**	**No. of resistant Klebsiella**	**Resistance %**	
Cephelexin	274	202	73.7	208	166	79.8	0.07
Gentamicin	224	118	52.7	207	118	57	0.21
Amikacin	118	32	27.1	98	28	28.6	0.46
Ciprofloxacin	105	**65**	61.9	94	70	**74.5**	**0.04**
Cefotaxime	27	18	66.7	46	36	78.3	0.2
Ceftriaxone	93	53	57	47	38	80.9	**0.003**
Cotrimoxazole	271	189	69.7	205	138	67.3	0.32
Nalidixic acid	304	205	67.4	93	66	71	0.3
Nitrofurantoin	347	198	57.1	105	60	57.1	0.53
Norfloxacin	322	164	50.9	208	127	60.6	**0.01**

All Klebsiella isolates were resistant to Ampicillin. There was significant increase in invitro resistance to quinolones and ceftriaxone. Except for Sulphonamides, there was an increase in resistance to other antibiotics over the years, although not statistically significant.

Table: 8 Isolates from Aspirates & Swabs

	Period A			Period B			
ANTIBIOTICS	**No. of isolates tested**	**No. of resistant Klebsiella**	**Resistance %**	**No. of isolates tested**	**No. of resistant Klebsiella**	**Resistance %**	**P value**
Cephelexin	84	63	75	121	104	86	**0.03**
Gentamicin	91	47	51.6	116	81	69.8	**0.005**
Amikacin	35	8	22.9	55	17	30.9	0.279
Ciprofloxacin	92	22	23.9	118	61	51.7	**0**
Cefotaxime	22	9	44.4	63	51	58.2	0.09
Ceftriaxone	45	20	40.9	79	46	81	**0**
Cotrimoxazole	90	56	62.2	109	72	66	0.339

A significant increase in resistance to 1st generation Cephalosporins, Gentamicin, Ciprofloxacin and Ceftiaxone was observed in the Period B compared to Period A

Table: 9 Blood isolates

	Period A			Period B			
ANTIBIOTICS	**No. of isolates tested**	**No. of resistant Klebsiella**	**Resistance %**	**No. of isolates tested**	**No. of resistant Klebsiella**	**Resistance %**	**P value**
Ampicillin	43	42	97.7	60	57	95	0.44
Cephelexin	28	26	92.9	60	55	91.7	0.6
Gentamicin	43	28	**65.1**	58	34	**58.6**	0.32
Amikacin	20	9	**45**	29	8	**27.6**	0.17
Ciprofloxacin	27	8	**29.6**	44	25	**56.8**	**0.022**
Ceftazidime	5	3	60	5	4	80	0.5
Cefotaxime	36	23	**63.9**	35	21	**60**	0.46
Ceftriaxone	25	13	52	29	21	72.4	0.1
Cotrimoxazole	31	20	64.5	60	40	66.7	0.5

A significant increase in resistance was noted for ciprofloxacin (from 29.6% to 56.8%).

Comparison of Resistance to Individual Antibiotics by Pseudomonas during Period A & Period B

Table: 10 Urine isolates

	Period A			Period B			
ANTIBIOTICS	**No. of isolates tested**	**No. of resistant Pseudomonas**	**Resistance %**	**No. of isolates tested**	**No. of resistant Pseudomonas**	**Resistance %**	**P value**
Gentamicin	29	16	55.2	30	23	76.7	0.07
Amikacin	23	7	30.4	31	19	61.3	**0.02**
Ciprofloxacin	26	15	57.7	32	23	71.9	0.19
Ceftazidime	6	3	50	24	12	50	0.67

Pseudomonas showed an increase in resistance to Amikacin, Gentamicin & Ciprofloxacin.

Table: 11 Isolates from Aspirates & Swabs

	Period A			Period B			
ANTIBIOTICS	**No. of isolates tested**	**No. of resistant Pseudomonas**	**Resistance %**	**No. of isolates tested**	**No. of resistant Pseudomonas**	**Resistance %**	**P value**
Gentamicin	124	66	53.2	128	84	65.6	**0.03**
Amikacin	112	30	26.8	130	44	33.8	0.147
Ciprofloxacin	127	37	29.1	139	60	43.2	**0.012**
Ceftazidime	21	15	71.4	66	26	39.4	**0.01**

A significant increase in resistance to Gentamicin & Ciprofloxacin is observed during the period B and also a statistically significant decrease in resistance to Ceftazidime. Resistance rate to Amikacin has not changed significantly.

Table: 12 Blood isolates

	Period A			Period B			
ANTIBIOTICS	**No. of isolates tested**	**No. of resistant Pseudomonas**	**Resistance %**	**No. of isolates tested**	**No. of resistant Pseudomonas**	**Resistance %**	**P value**
Gentamicin	21	12	57.1	14	7	50	0.47
Amikacin	17	6	35.3	11	7	53.6	0.14
Ciprofloxacin	18	2	11.1	15	4	26.7	0.24
Ceftazidime	14	6	42.9	7	4	57.1	0.43

No significant change in resistance pattern is observed with Pseudomonas spp.

Resistance Rate of Other GNB during Period A & Period B

GNB other than E.coli, Klebsiella and Pseudomonas are considered together, which include Coliforms, Proteus, Acinetobacter, Flavobacteria, S.paratyphi, S.typhi, Enterobacter & Alkaligens faecalis. Isolates from urine showed no significant change in resistance to the antibiotics tested over the study periods. Isolates from aspirates and swabs showed significantly increased Resistance to Ciprofloxacin in period B and resistance to Amikacin, Cefotaxime & Cotrimoxazole was found to have decreased.

Multidrug Resistant Gram Negative Bacteria

A significant increase in Multidrug resistant strains of E.coli, Klebsiella and Pseudomonas was observed during Period B, compared to period A (Table 13, 14, 15).

Table: 13 MDR GNB – urine isolates

Year	**E.coli**		**Klebsiella**		**Others**		**Pseudomonas**	
	No. of MDR isolates	Percent. %	No. of MDR isolates	Percent. %	No. of MDR isolates	Percent. %	No. of MDR isolates	Percent.
Period A	33	5	26	6.9	10	5.3	2	6.3
Period B	94	13.3	36	14.1	1	3.1	11	30.6
TOTAL	127		62		11		13	
p value		**5.05E-08**		**0.00262**		0.50294		**0.01085**

Table: 14 MDR GNB isolates from aspirates and swabs

Year	**E.coli**		**Klebsiella**		**Others**		**Pseudomonas**	
	No.of MDR isolates	**Percent.**	**No.of MDR isolates**	**Percent.**	**No.of MDR isolates**	**Percent.**	**No.of MDR isolates**	**Percent.**
Period A	12	17.6	11	10.6	8	10.7	0	0
Period B	30	27.8	41	29.1	13	20.6	4	25
TOTAL	42		52		21		4	
p value		0.086696		**0.0002964**		0.0830222		**0.022**

Table: 15 MDR GNB isolates from blood

Year	**E.coli**		**Klebsiella**		**Others**		**Pseudomonas**	
	No. of MDR isolates	Percent.	No. of MDR isolates	Percent.	No. of MDR isolates	Percent.	No. of MDR isolates	Percent.
Period A	3	18.8	6	12.5	11	9.7	0	0
Period B	7	36.8	14	20.3	2	4.3	4	25
TOTAL	10		20		13		4	
p value		0.211666		0.198372		0.205114		**0.022127**

There was a significant increase in number of MDR Pseudomonas isolates during the period B

Discussion & Conclusions

It is widely accepted that monitoring locally generated trends of antimicrobial resistance is important as an aid to clinical decision making and development of infection control and resistance containment strategies. This study analysed and compared the profile and ABST patterns of the Gram negative bacterial isolates obtained from clinical materials in a tertiary care centre hospital laboratory over a period of 8 years, dividing the study period into 2 groups, period A & Period B, each holding 4 years. For E.coli and Klebsiella urine isolates, a significant increase in resistance to quinolones and 3rd Generation Cephalosporin was observed over the years. Although the resistance rates of Urine E coli to Nitrofurantoin and Amikacin were found to be increasing , that was not found to be statistically significant. Pseudomonas aeruginosa and Klebsiella spp , the commonest GNB isolates from swabs and body fluids, may be considered as the predominant hospital flora as majority of samples tested were from hospitalised patients. For Pseudomonas, a statistically significant decrease in resistance to Ceftazidime and a significant increase in resistance to Gentamicin and Ciprofloxacin was documented during Period B. A similar trend of increasing resistance between study periods was documented in a study conducted by Neuromicrobiology Department, NIMHANS, Bangalore.(12) Acinetobacter, the commonest blood isolate during the period A showed a shift during the second period wherein Klebsiella became predominant. The commonest isolate from the pediatric age group was Klebsiella. This is in variance to reports from around the world where E.coli is the most common blood isolate GNB. (9), (10), (11) Klebsiella blood isolates have shown a trend of decreasing resistance to Aminoglycosides-Gentamicin and Amikacin– , but statistical significance was not demonstrated. On the other hand a significant increase in resistance to Ciprofloxacin and 3rd generation Cephalosporins was noticed. Data from the current study indicates a benefit of Aminoglycoside antibiotics especially Amikacin for empirical therapy of suspected Gram negative bacterial sepsis.

The broad trends that have emerged from the current study include:

1. A near universal resistance to Ampicillin for almost all GNB isolated regardless of the site of isolation, bringing into question the very rationale of continued testing for Ampicillin sensitivity for Gram negative bacteria
2. The high resistance documented to commonly used Cephalosporins- Ceftriaxone and Cefotaxime for most GNB isolates from the different sites
3. The increasing isolation rate of Multi drug resistant strains.

In the present scenario where ESBLs , MDR and XDR GNB strains have emerged and posing great challenge in the treatment especially in tertiary care/Intensive care settings and serious consideration of antibiotic rotation and cycling are on process, which requires reviews and standardization[13] this study may be used for a baseline references.

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