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Prevalence of Gram-negative bacteria and their antibiotic-resistance pattern at tertiary care hospital Amreli Gujarat India

***Sanjeev K., Krina V.**

Abstract

Background: Infections with multi-drug resistant (MDR) bacteria are serious threats to healthcare services many low-income countries associated with overuse and misuse of antibiotics.

Material and Methods: A prospective cross-sectional study was done between January, 2021 to December, 2021 at Shantabaa Medical College and General Hospital, Amreli, Gujarat, India. Clinical specimens such as swab from wound, urine, sputum, blood, body fluids and throat swab were sampled following standard operating procedures.

Results: A total of 738 patient specimens were collected and processed from different clinical samples such as urine 540 (73%), wound swab 48 (6.5%), sputum 96 (13%), blood 06 (1%), throat swab 12 (1.5%) and other body fluids 36 (5%). Overall, 142 (19.2%) of the specimen collected were culture positive. From the 142 positive patients, the proportion of bacterial isolates was higher in females 95 (67%), than males 47 (33%). Out of 142 culture positive isolates, *Eshcerchia coli (E.coli)* 56 (39%), followed by *Pseudomonas aeruginosa* 35 (25%), *Klebsiellae* 24 (17%), *Enterobacter* 12 (8%), *Citrobacter* 8 (6%), *Acinetobacter* 5 (4%) and *Serratia* 2 (1%).

Conclusion: *E.coli* followed by *Pseudomonas aeruginosa*, *Klebsiellae*, and *Enterobacter* were the most commonly isolated gram-negative bacteria. These bacteria were resistant to the routinely used antibiotics. Therefore, clinicians should practice rational choice of antibiotics and treatment should be guided by antimicrobial susceptibility testing.

Keywords: Gram-negative bacteria, clinical samples, antibiotic resistant pattern, multi-drug resistant.

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Prévalence des bactéries gram-négatives et leur profil de résistance aux antibiotiques à l'hôpital de soins tertiaires amreli gujarat inde

***Sanjeev K., Krina V.**

Résumé

Contexte général de l'étude : Les infections par des bactéries multirésistantes (IBM) constituent de graves menaces pour les services de santé de nombreux pays à faible revenu, associées à la surutilisation et à l'abus d'antibiotiques.

Matériel et méthode de l'étude : Une étude transversale prospective a été réalisée entre janvier 2021 et décembre 2021 au Shantabaa Medical College and General Hospital, Amreli, Gujarat, Inde. Des échantillons cliniques tels que des écouvillons de plaies, d'urine, de crachats, de sang, de liquides organiques et d'écouvillons de gorge ont été prélevés conformément aux procédures opératoires standard.

Résultat de l'étude : Un total de 738 échantillons de patients ont été collectés et traités à partir de différents échantillons cliniques tels que l'urine 540 (73 %), l'écouvillonnage de la plaie 48 (6,5 %), les expectorations 96 (13 %), le sang 06 (1 %), l'écouvillonnage de la gorge 12 (1,5 %) et autres fluides corporels 36 (5 %). Dans l'ensemble, 142 (19,2 %) des échantillons prélevés étaient positifs à la culture. Parmi les 142 patients positifs, la proportion d'isolats bactériens était plus élevée chez les femmes 95 (67 %), que chez les hommes 47 (33 %). Sur 142 isolats positifs à la culture, *Eshcerchia coli (E. coli)* 56 (39 %), suivi de *Pseudomonas aeruginosa* 35 (25 %), *Klebsiellae* 24 (17 %), *Enterobacter* 12 (8 %), *Citrobacter* 8 (6 %), *Acinetobacter* 5 (4 %) et *Serratia* 2 (1 %).

Conclusion: *E. coli* suivi de *Pseudomonas aeruginosa*, *Klebsiellae* et *Enterobacter*étaient les bactéries gram-négatives les plus fréquemment isolées. Ces bactéries étaient résistantes aux antibiotiques couramment utilisés. Par conséquent, les cliniciens doivent pratiquer le choix rationnel des antibiotiques et le traitement doit être guidé par des tests de sensibilité aux antimicrobiens.

Mots-clés: Bactéries gram-négatives, échantillons cliniques, antibiorésistance, multi-résistance aux médicaments.

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INTRODUCTION

Infections caused by gram-negative bacteria include meningitis, urinary tract infections, pneumonia, blood stream infections, wound and surgical site infections in medical settings. [1]. Despite the development of broad range antibiotics over the past 70 years, these life-threatening bacteria continue to cause a high rate of mortality and morbidity. This is a result of gram-negative bacteria developing an increased rate of antibiotic resistance [2]. One of the major risks to the world's public health is antimicrobial resistance (AMR), which makes it difficult to treat infections effectively and increases the likelihood that patients may have severe sickness, complications, and even death. According to estimates, AMR currently claims 700,000 lives each year, and if nothing is done, that number would increase to 10 million by the year 2050 [3]. Due to the illogical prescription and/or inappropriate usage of antibiotics, AMR has significantly increased [2,4].

In order to tackle the AMR epidemic, encourage the development of novel medicines, and promote the prudent use of that already in use, the World Health Organization (WHO) published a list of antibiotic-resistant priority infections. Antibiotic resistance evolves when the bacteria can escape the effect of antibiotics by different mechanisms, like the neutralizing the antibiotics, pumping them outside of the cell or modifying their outer structure resulting in inhibition of the drugs' attachment to the bacteria and DNA transfer through a horizontal gene transfer between bacteria via transformation or transduction or by conjugation. The majority of these microorganisms are gram-negative bacteria [5,28]. *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterobacter*, *Citrobacter, Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter* spp. are the commonly isolated organisms from infections in the clinical and community settings. They are also the current most serious antibiotic-resistant organisms [8, 9].

Drug-resistant pathogen infections have a profound impact on both the economic stability of societies and public health globally. Clinical infections with drug-resistant organisms continue to be important causes of morbidity and mortality in hospitalized patients and in community settings, impacting both developed and middle-income nations, despite major advancements in infection control methods [6,7]. Multi-drug resistant pathogens are a common cause of hospital associated infections (HAIs) and a place a heavy toll on patients and their families by causing illness, potential disability, excess cost and sometimes death [29]. The study's goal is to determine the prevalence and antibiogram of gram-negative bacteria in various clinical samples at our healthcare centre.

MATERIALAND METHODS

A prospective cross-sectional study was done between January, 2021 to December, 2021 at Shantabaa Medical College & General Hospital, Amreli, Gujarat, India. Clinical specimens such as swab from wound, urine, sputum, blood, body fluids and throat swab were sampled following standard operating procedures. Depending on the source of the specimen, each sample was plated on Blood agar, Macconkey's agar, CLED agar, Nutrient agar and Chocolate. All the inoculated plates were incubated aerobically at 37^0C for 24-48 hrs. Bacterial isolates were identified by standard phenotypic microbiological methods [10,11].

Antimicrobial susceptibility test:

Susceptibility of bacterial isolates to different antibiotics was analyzed by Kirby–Bauer disk diffusion susceptibility testing on Muller Hinton Agar (MHA). All the identified bacterial isolates were checked for susceptibility to Ampicillin (10 µg), Cefoxitin (30 µg),Ttetracycline (30 µg), Nitrofurantoin (300 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Tobramycin (10 µg), Amikacin (30 µg), Ampicillin/Sulbactam (10/10 µg), Piperacillin/tazobactam (100/10 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Cotrimoxazole (25 µg), Trimethoprim (5 µg), Norfloxacin (10 µg), Fosfomycin (200 µg), Cefepime (30 µg), Cefotaxime (30 µg), Aztreonam (30 µg), Imipenem (10 µg) and Meropenem (10 µg).

The choice of antibiotic agents is based on the commonly available drugs and drugs which are frequently prescribed by physicians. Resistance data were interpreted according to zone sizes from the Clinical and Laboratory Standards Institute (CLSI) guideline. American Type Culture Collection (ATCC) standard reference strain (*E. coli* ATCC-25922 was used to verify the performance of the culture media and antibiotics [12].

RESULTS

A total of 738 patient specimens were collected and processed from different clinical samples such as urine 540 (73%), wound swab 48

(6.5%), sputum 96 (13%), blood 06 (1%), throat swab 12 (1.5%) and other body fluids 36 (5%). Overall, 142 (19.2%) of the specimen collected were culture positive. From the 142 positive patients, the proportion of bacterial isolates was significantly higher in females 95 (67%), than males 47 (33%).

Out of 142 culture positive isolates, *Eshcerchia coli (E.coli)* 56(39%), followed by *Pseudomonas aeruginosa* 35 (25%), *Klebsiellae* 24 (17%), *Enterobacter* 12 (8%), *Citrobacter* 8 (6%), *Acinetobacter* 5 (4%) and *Serratia* 2 (1%) [Chart 1].

DISCUSSION

The prevalence of culture confirmed gram-negative bacterial infections was higher in females than males in the present study. The finding is in correlates with study of Rossi F [13] and Yitayeh et al [14]. This may be due to large number of isolates were isolated form urinary tract infections (UTI) and females are more prone to get UTI [15]. In this study, *E. coli* was the most predominant isolate. This is similar to studies from Burkina Faso [16] and Kigali, Rwanda [17] and studies from other parts of Ethiopia [15, 18]. This could be due to the abundance of *E. coli* in urinary tract infection and its virulence factors and interaction with the host.

P.aeruginosa has been emerged as a significant pathogen and is the most common dreadful gram negative bacilli found in various health care associated infections all over the world due to its virulence, well known ability to resist killing by various antibiotics and disinfectants. In the present study we have isolated 25% of P.aeruginosa from various clinical specimens. Although in India, prevalence rate of P.aeruginosa infection varies from 10.5% to 30%. Tadvi J et al. reported the prevalence rate of P.aeruginosa was 4.15% [19]. In our study ciprofloxacin and levofloxacin showed 40% resistant against P.aeruginosa. In various reports on ciprofloxacin resistance to P.aeruginosa was ranged between 0-89% [20].

Klebsiella pneumonia is worldwide one of the most frequently isolated gram negative bacteria from clinical specimens of hospitalized patients. There has been an increase in the incidence of infections with these bacteria during the past decade, probably due to an increase in the number of hospitalized or immune-compromised individuals. Also a trend towards greater antibiotic resistance is seen [21,22]. The present study highlights an alarming situation of high antibiotic resistance among the isolates of *Klebsiella pneumonia*. In the present study 100% isolates showed resistance to Ampicillin. The cause may be chromosomally encoded β-lactamases responsible for intrinsic resistance [23].

Enterobacter has become increasingly resistant to many previously effective antibiotics. In 2017, the World Health Organization issued a list of antibiotic resistant bacteria in which carbapenem reistant Enterobacteriaceae was in the critical priority group for an urgent needs to develop new antibiotics [24].

Multidrug resistant (MDR) Acinetobacter baumannii is becoming a global threat with a therapeutic impasse increasingly described in literature [25]. According to the literature data, the resistance rate varies from 31.8 to 92.1% against ceftazidime; 8.8 to 89.9% against imipenem, from 28.8 to 91.6% against fluoroquinolones and 30 to 90% vs aminoglycosides [26]. In the present study Acinetobacter showed 80% resistant to fluoroquinolones and 60% resistant to aminoglycosides. However Acinetobacter is an opportunistic pathogen known for its intrinsic resistance to antibiotics and greater ability to rapidly acquire resistance genes as mobile genetic elments [27].

CONCLUSION

E.coli, P.aeruginosa, Klebsiellae and Enterobacter spp. were the predominant gram-negative bacteria isolated from different clinical samples. Most of the clinical isolates were resistant to ampicillin, co-trimoxizole, fluoroquinolones, tetracycline and cephalosporins. Therefore it is recommended to have strict antibiotic utilization policies within the hospital, regular surveillance of antibiotic sensitivity pattern among the clinical isolates and to support clinicians on rational choice of antibiotics therapy.

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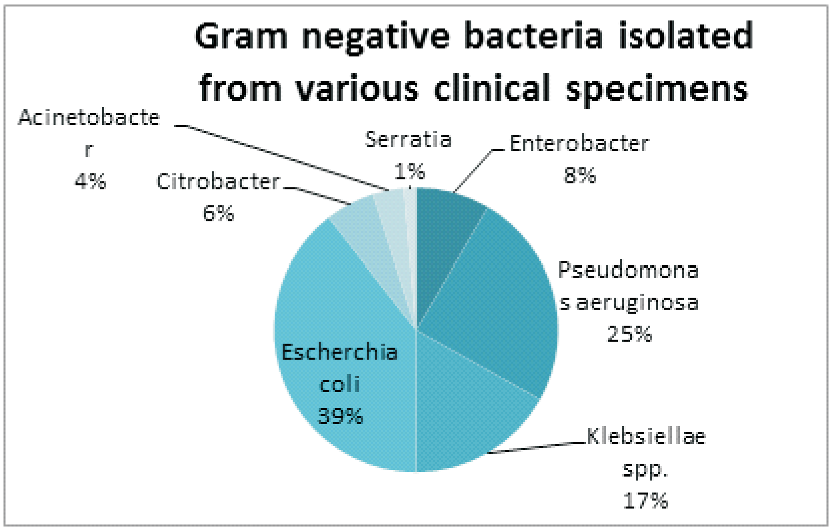

E.coli isolates showed 86% resistant to ampicillin and 57% resistant to co-trimoxizole, cefuroxime, cefipime and tetracycline. *Pseudomonas aeruginosa* showed 40% resistant to levofloxacin, ciprofloxacin and cefipime. *Klebsiellae* isolates showed 100% resistant to Ampicillin, cefuroxime and cefipime. (Table 1)

Table 1: Antibiotic Resistance pattern of Gram negative bacteria

Organism (No.of isolates = n)	**The following antibiotics showing Resistance (R) against the number of isolates**																					
	GENTAMICIN	**TOBRAMYCIN**	**AMIKACIN**	**AMPICILLIN/SULBACTUM**	**PIPERACILLIN/TAZOBACTAM**	**CEFUROXIME**	**CEFIPIME**	**CEFOXITIN**	**CEFTRIOXONE**	**CIPROFLOXACIN**	**LEVOFLOXACIN**	**MEROPENAM**	**COTRIMOXIZOLE**	**AZTREONAM**	**CEFTAZADIME**	**CHLORAMPJENICOL**	**TETRACYCLINE**	**CEFAZOLIN**	**FOSFOMYCIN**	**NITROFURONTOIN**	**AMPICILLIN**	**MINOCYCLIN**
Escherichia coli (n=56)	8	8	10	22	10	32	29	18	19	24	26	2	31	14	18	16	32	12	6	2	48	-
Pseudomonas aeruginosa (n=35)	8	8	6	-	7	-	14	-	-	12	14	1	-	5	8	-	-	-	-	-	-	-
Klebsiellae spp. (n=24)	7	6	4	9	5	24	24	11	15	10	9	3	10	10	14	7	16	7	0	0	24	-
Enterobacter (n=12)	3	3	2	5	4	8	8	8	8	6	9	0	7	9	8	10	6	12	0	0	10	-
Citrobacter (n=8)	2	2	2	-	-	2	4	2	6	2	2	-	6	6	6	-	4	2	-	-	4	-
Acinetobacter (n=5)	-	-	0	2	-	1	1	1	4	3	3	-	2	1	4	-	1	-	-	-	1	1
Serratia (n=1)	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	1	1	-	-	-	1	-