Original Article:

Prevalence of imipenem resistant gram-negative bacteria in a tertiary care hospital of Dhaka, Bangladesh

Rabeya Nahar Ferdous[1], Md. Atikur Rahman[2], Md. Anowar Hussain[3], Nasrin Akhter[4], Palash Chandra Banik[5], Mohammad Mahmudur Rahman[6]

Abstract

Objective: Imipenem resistant gram-negative bacteria (GNB) have become a major public health concern worldwide, including Bangladesh. The present study was performed to determine the frequency of imipenem resistant gram-negative bacteria (GNB), their antimicrobial susceptibility pattern. ***Materials and Methods:*** A total of three hundred and fifty clinical samples were collected from Bangladesh Institute of Health Sciences hospital (BIHS), Dhaka, Bangladesh, over a period of 12 months. Among 350 samples, 171 (48.86%) were from indoor patients, and 179 (51.14%) were from outdoor patients. The pathogens were isolated and identified by conventional methods and were screened for antibiotic susceptibility using the Kirby–Bauer disc diffusion method, including imipenem discs. A Chi-square test was employed for statistical analysis. ***Results and Discussion:*** Out of 350 clinical isolates, 246 showed resistance to imipenem (70.28%). Almost all of the imipenem resistant gram-negative bacteria showed the highest resistant pattern to cefepime (88.57%), amoxicillin (88.29%), cephalosporin (88.14%), cefoxitin (86%), tetracycline (84.42%), and the majority were resistant to levofloxacin (70.85%), doxycycline (70.57%), netilmicin (59.71%). But cotrimoxazole (13.42%) and tigecycline (11.43%) showed a lower resistance pattern. Statistical analysis exhibited imipenem resistant gram-negative isolates most commonly found in pus and urine samples, while *Klebsiella spp* (30.49%), *Pseudomonas spp* (26.83%) and *E. coli* (23.17%) were the most predominant pathogens. ***Conclusion:*** This is a retrospective study which study indicates a noteworthy rate of clinical isolates were imipenem resistant gram-negative bacteria in a well-defined tertiary care hospital, and most of these bacteria were also multidrug-resistant.

Keywords: Imipenem resistance; multidrug-resistance; tertiary care hospital; gram-negative bacteria (GNB); Bangladesh

Bangladesh Journal of Medical Science Vol. 21 No. 01 January'22 Page : 145-150
DOI: https://doi.org/10.3329/bjms.v21i1.56341

Introduction

Carbapenems, including imipenem and meropenem, are the most potent antibacterial agents used for the treatment of infections caused by multidrug-resistant gram-negative bacteria[1]. Although carbapenems remain effective in the dealing of Gram-negative pathogens, high carbapenem resistance rates in Gram-negative bacteria including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* have emerged and distributed globally[2,3].

Detection of imipenem resistance is a crucial infection control issue because they are often associated with widespread antibiotic resistance in hospitals, long-term acute care hospitals and long-term care facilities[4]. The prevalence of imipenem resistance in Gram-negative bacteria (GNB) has increased markedly in the earlier years[5,6]. Imipenem

1. Rabeya Nahar Ferdous, Department of Microbiology, Bangladesh University of Health Sciences.
2. Md. Atikur Rahman , Institute of Microbiology, Friedrich-Schiller-University Jena, Germany.
3. Md. Anowar Hussain, Department of Community Dentistry, Bangladesh University of Health Sciences.
4. Nasrin Akhter , Department of Pharmaceuticals Sciences, North South University.
5. Palash Chandra Banik, Department of Non-Communicable Disease, Bangladesh University. of Health Sciences.
6. Mohammad Mahmudur Rahman, Department of Medical Biotechnology, Bangladesh University of Health Sciences.

Correspondence: Rabeya Nahar Ferdous**,** Department of Microbiology, Bangladesh University of Health Sciences. E-mail: rabeya.nahar007@gmail.com;

resistant Gram-negative bacteria (GNB) are reported as important causes of hospital-acquired infections[5]. Worldwide, imipenem resistant gram-negative bacteria increasing day by day due to inappropriate and indiscriminate use of antibiotics, crowding of patients with high levels of disease acuity in relatively small, specialized areas of the hospital, shortage of nursing and other supporting staff due to economic pressures (which increases the likelihood of person-to-person transmission of microorganisms) and the presence of more chronically and acutely ill patients who require prolonged hospitalization[7,8]. The frequency of imipenem resistance remains doubtful due to the absence of proper detection techniques in many countries, particularly those with inadequate resources and poor laboratory settings[9]. Moreover, antibiotics are prescribed prophylactically and empirically without carrying out sensitivity studies mostly in developing countries. In developing countries, several other factors are mainly involved in antibiotic resistance such as antibiotics of low quality, lack or improper antibiotic resistance surveillance, and easy accessibility of antibiotics[10].

The prevalence of carbapenems, including imipenem resistant gram-negative bacteria isolated from patients in Bangladesh, has been reported[11-13]. A recent study in Bangladesh shows that the emergence rate of imipenem resistant gram-negative urinary isolates is 14.49%[11]**.** Notably, the early detection of carbapenemase-producing Gram-negative bacteria in their primitive infection stage is essential to prevent the progression of hospital-based outbreaks[14]**.** So, diagnostic surveillance is becoming necessary in healthcare institutions to reduce infection rates and to better understand the disease.

The objective of this present study was to determine the frequency of imipenem resistants among gram-negative bacteria isolated from different clinical samples.

Methods

Sample collection

It was a retrospective study. Three hundred and fifty clinical samples were collected during the months from January to December 2019 from Bangladesh Institute of Health Sciences (BIHS), Dhaka, Bangladesh. (Table 1).

Table 1: Sample size and type

Sample types	No. of samples
Pus	147
Urine	133
Wound swab (w/s)	51
Sputum	11
High vaginal Swab (h/v)	08

Isolation and identification of gram-negative bacteria

The samples were cultured in respective media for isolation of potential pathogens and then identified by cultural characteristics, colony morphology, gram staining, and biochemical tests[15].

Antimicrobial susceptibility test

Antimicrobial susceptibility pattern was determined in vitro by using the standardized agar-disc-diffusion method known as Kirbey-Baur disk diffusion method[16]. The antibiotics were tested as follows (potency µg/disk): Amoxicillin (30), cefepime (30), cefoxitin (30), cephalosporin (30), cotrimoxazole (25), doxycycline (30), imipenem (10), levofloxacin (5), netilmicin (30), tetracycline (30), tigecycline (15).

Screening for imipenem resistance by the disc diffusion technique

Screening for imipenem -resistance was determined using the Kirbye-Bauer disc diffusion method with a 10 µg imipenem disc. Three pure colonies of test organisms were inoculated into 3 mL of sterile normal saline. The turbidity of the suspension was compared with the 0.5 McFarland turbidity standard, and the suspension was incubated on Muellere-Hinton agar plates at 37°C for 24 hours. An inhibition zone of ≤19mm diameter around the imipenem disc was considered resistant[17]**.**

Data Analysis

Data were analyzed using SPSS software version 23. Descriptive data was presented to the table as number and frequency. The Chi-square test was performed to see the association.

Ethical clearance: This study was approved by ethics committee of Bangladesh University of Health Sciences.

Results

Three hundred and fifty clinical samples (pus, urine, sputum, wound swab, high vaginal Swab) were collected from patients (164 male and 186 female) (Table 2). Age and sex distribution patterns of their samples showed that most of the samples were collected from female gender and their age group was between 51–60 years were more susceptible to cause infection.

Table 2: Age and Sex distribution of patients

Age	Male (%) n= 164	Female (%) n= 186
11-20	0 (0)	01 (0.29)
21-30	03 (0.86)	13 (3.71)
31-40	14 (4)	21 (6)
41-50	40 (11.43)	45 (12.86)
51-60	48 (13.71)	50 (14.29)
61-70	41 (11.71)	36 (10.29)
71-80	15 (4.29)	18 (5.14)
≥80	03 (0.86)	02 (0.57)

Isolation and identification

Among 350 isolates 294 confirmed as gram-negative bacteria by cultural characteristics, colony morphology, gram's staining and biochemical tests.

Table 3: Type of isolated organisms with their occurrence number

Type of Gram-negative Organisms	Number of Gram-negative Organisms (n=294)
Klebsiella spp	87
Pseudomonas spp	69
E. coli	65
Acinetobacter Spp	23
Proteus Spp	19
Enterobacter Spp	31

Antibiotic susceptibility pattern

Two hundred and forty-six of the total 294 Gram-negative isolates (70.28%) were found to be imipenem resistant by the disk diffusion test. Isolates were also tested against ten commonly used antibiotics. Isolated strain showed highest resistance to cefepime (88.57%), followed by amoxicillin (88.29%), tetracycline (84.42%), cephalosporin (88.14%), cefoxitin (86%). Furthermore, isolates were found to be resistant to doxycycline (70.57%), levofloxacin (70.85%), netilmicin (59.71%). Cotrimoxazole (13.42%) and tigecyline (11.43%) showed lowest resistant among other antibiotics.

Data analysis

Chi-square test was employed by using SPSS version 23.0 to compare the categorical variables between the two groups. $P < 0.050$ was considered statistically significant. Out of 246 carbapenemase producing gram-negative, where 125 from indoor patients (IPD) and 121 outdoor patients (OPD) were studied. Statistical analysis was performed between the type of infection and patient type (IPD and OPD). Statistical analysis results significant association between clinical samples and type of patients. For the detection of carbapenemase producing gram-negative bacteria, pus found as a good source for indoor patients, and urine found as a good source for outdoor patients (Table 4). The association was found between sample type and type of carbapenemase producing gram-negative pathogenic bacteria (*Klebsiella spp*, *Pseudomonas spp*, *E. coli, Acinetobacter, Proteus,* and *Enterobacter*). According to this analysis *Klebsiella spp, Pseudomonas spp*, and *E.coli* those are most prominent pathogenic organisms which are responsible for producing carbapenemase. But in the case of Acinetobacter, Proteus, Enterobacter there is no association with sample type to produce carbapenemase (Table 5).

Table 4: Association between sample type and patient type

Sample Type	IPD (n=125)	OPD (n=121)	χ^2 value	P-value
Pus	58	38	12.95	0.02
Urine	34	59	13.28	0.01
Wound Swab	26	20	0.84	0.92
Sputum	04	03	5.78	0.375
High vaginal swab	03	01	0.95	0.925

$P < 0.050$ considered statistically significant

Table 5: Association between the type of carbapenemase producing pathogenic gram-negative bacteria and sample type

Type of carbapenamse producing pathogenic gram-negative bacteria	Sample Type					χ^2 value	*P* Value
	Pus	Urine	Wound Swab (w/s)	Sputum	High Vaginal Swab (H/V)		
Klebsiella spp	27	32	11	03	02	33.67	0.03
Pseudomonas spp	25	23	17	01	-	_	000
E.coli	24	31	-	-	02	_	000
Acinetobacter	03	01	12	01	-	29.30	0.175
Proteus spp	05	02	02	-	-	37.57	0.10
Enterobacter spp	09	07	02	04	-	18.23	0.60

P < 0.050 is considered statistically significant.

Discussion

In recent years, through the abuse and misuse of antibiotics, many bacteria have developed resistance to the variety of antibiotics including imipenem. Imipenem is a potent beta-lactam antibiotic[18]. Increasing the resistant rate of imipenem is a major public health problem in all populations. The pattern of resistance can be different in various populations because imipenem is one of the most commonly used antibiotics. So it is necessary to introduce to a special program for reduction of resistance to this antibiotic. Continuous monitoring of these virulent organisms may check the spread and play a vital in controlling the infection.

In this study, total 350 samples (pus, urine, wound swab, sputum, high vaginal Swab) from patients were collected from BIHS located in Mirpur, Dhaka of which majority of the patients were female (53.15%), which found similar to the report in India[19]. Patients from the age group 51-60 years contributed (28%) but in the previous studies age group was found between 21-40 in Pakistan and Bangladesh also[20-22]

The present study identified 294 gram-negative bacteria where *Klebsiella spp, Pseudomonas spp, E. coli, Acinetobacter Spp, Proteus spp, Enterobacter spp*were found by colony morphology, microscopy and relevant biochemical tests[15].

The antibiotics selected for this study are Amoxicillin, cefepime, cefoxitin, chlorpromazine, cotrimoxazole, doxycycline, imipenem, levofloxacin, netilmicin, tetracycline, tigecycline. Out of 294 gram-negative bacteria246 (70.28%) confirmed as carbapenemase producer by measuring zone of inhibition ≤19mm diameter around the imipenem (10 µg) disc[17]. Among 246 (70.28%) carbapenemase-producing gram-negative bacteria most of them found in pus 39.02%, urine 37.80%, wound swab 10.65%, sputum 2.85%, high vaginal Swab 1.62% respectively. A previous study showed carbapenemase-producing gram-negative bacteria was only 14.29% from urinary isolates which is increasing day by day [(11)]. Almost all of the imipenem resistant carbapenemase producing gram-negative bacteria showed the highest resistant pattern to cefepime (88.57%), amoxicillin (88.29%), tetracycline (84.42%), chlorpromazine (88.14%), cefoxitin (86%), majority were resistant to doxycycline (70.57%), levofloxacin (70.85%), and netilmicin (59.71%). But cotrimoxazole (13.42%) and tigecycline (11.43%) showed lower resistant pattern.

In this study among 70.28% imipenem resistant gram-negative bacteria *Klebsiella spp* (30.49%), *Pseudomonas spp* (26.83%), *E. coli* (23.17%)**,** *Enterobacter spp (8.94%), Acinetobacter spp* (6.91%), *Proteus spp* (3.66%)were increasing over time, where Ahmed *et al* reported 2.3% *E. coli*, 13.5% *Pseudomonas spp,* 0% *Klebsiellaspp* and *Acinetobacter spp* in 2018 [(23)]. But no extended work found in the case of *Enterobacter spp* and *Proteus spp*. According to the chi-square test, data analysis showed imipenem resistant isolates most commonly found in pus and urine where, *Klebsiella spp* (30.49%), *Pseudomonas spp* (26.83%),*E. coli* (23.17%) found as the most predominant pathogen.

The study provides information about imipenem resistant gram-negative bacteria and their pattern of resistance. The prevalence of imipenem resistant gram-negative bacteria bring large financial burden to healthcare facilities and patients due to its indiscriminate use. Every strain of bacteria is susceptible to a specific antibiotic; hence, it is important to identify the antibiotics before prescribing to the infected person. Due to lower in resistant pattern cotrimoxazole (13.42%) and tigecyline (11.43%) can be a drug of choice but it showed proper identification of resistant pattern before prescribing.

Finally, to reduce the rate of infection associated with imipenem resistance effort should be made for routine microbiological surveillance to reduce infections. It is also necessary to carry out a large-scale study with a combination of introduced drugs, which may help in our economy and as well as newer antimicrobials. This will hopefully reduce the resistance pattern and thus the treatment cost, and initiate quality patient care.

Conclusion

This retrospective study revealed that the prevalence of imipenem resistance is 70.28% which is detected by the zone of inhibition. People, age between (51- 60) most commonly associated with this resistant pattern. Among 246 (70.28%) carbapenemase-producing gram-negative bacteria most were found in the pus samples (39.02%). Among other gram-negative bacteria that are identified in this study *Klebsiella spp* (30.49%) found as highest one which is a major burden of disease. To have a substantial impact on the burden of imipenem resistance and to prevent this increasing incidence, risk factors associated with this pattern of resistance must be identified.

Declarations

Author contribution statement

Rabeya Nahar Ferdous, Md. Anowar Hussain, Nasrin Akhter, Mohammad Mahmudur Rahman: Conceived and designed the experiments; performed the experiments.

Rabeya Nahar Ferdous, Md. Atikur Rahman, Palash Chandra Banik: Analyzed and interpreted the data; drafted the manuscript.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgement

The experiment was performed in the Department of Microbiology, Bangladesh University of Health Sciences. We would like to give thanks the Department for all facilities.

References

1. Giamarellou, H., and Poulakou, G. Multidrug-resistant gram-negative infections. *Drugs*, 2009; **69**(14), 1879-1901.
2. Potron, A., Poirel, L., and Nordmann, P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. *International journal of antimicrobial agents*, 2015; **45**(6), 568-585.
3. Wang, J. T., Wu, U. I., Lauderdale, T. L. Y., Chen, M. C., Li, S. Y., Hsu, L. Y., and Chang, S. C. Carbapenem-nonsusceptible enterobacteriaceae in Taiwan. *PloS one*, 2015; 10(3), e0121668.
4. Perez, F., Endimiani, A., Ray, A. J., Decker, B. K., Wallace, C. J., Hujer, K. M., ... & Windau, A. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of post-acute care facilities on dissemination. *Journal of antimicrobial Chemotherapy*, 2010; **65(**8), 1807-1818.
5. Thomson, K. S. Extended-spectrum-β-lactamase, AmpC, and carbapenemase issues. *Journal of clinical microbiology*, 2010; **48**(4), 1019-1025.
6. Nordmann, P., Naas, T., and Poirel, L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerging infectious diseases*, 2011; **17**(10), 1791.
7. Kollef, M. H., and Fraser, V. J. Antibiotic resistance in the intensive care unit. *Annals of internal medicine*, 2001; **134**(4), 298-314.
8. Shankar, P. R., Partha, P., Dubey, A. K., Mishra, P., and Deshpande, V. Y. Intensive care unit drug utilization in a teaching hospital in Nepal. *Kathmandu University medical journal (KUMJ)*, 2005; **3**(2), 130.
9. Jean, S. S., and Hsueh, P. R. High burden of antimicrobial resistance in Asia. *International journal of antimicrobial agents*, 2011***; 37(***4), 291-295.
10. Ahmed, I., Rabbi, M. B., and Sultana, S. Antibiotic resistance in Bangladesh: A systematic review. *International Journal of Infectious Diseases*, 2019***; 80,*** 54-61.
11. Begum, N., and Shamsuzzaman, S. M. Emergence of carbapenemase-producing urinary isolates at a tertiary care hospital in Dhaka, Bangladesh. *Tzu Chi Medical Journal*, 2016; **28(**3), 94-98.
12. Stewardson AJ, Marimuthu K, Sengupta S, Allignol A, El-Bouseary M, Carvalho MJ, Hassan B., et al. Effect of carbapenem resistance on outcomes of bloodstream infection caused by Enterobacteriaceae in low-income and middle-income countries (PANORAMA): a multinational prospective cohort study. *The Lancet Infectious Diseases***, 2019**; **19**(6), 601-610.
13. Islam, M. A., Talukdar, P. K., Hoque, A., Huq, M., Nabi, A., Ahmed, D., Endtz, H. P., et al. Emergence of multidrug-resistant NDM-1-producing Gram-negative bacteria in Bangladesh. *European journal of clinical microbiology & infectious diseases*, 2012***;*** **31**(10), 2593-2600.
14. Falagas, M. E., Karageorgopoulos, D. E., and Nordmann, P. Therapeutic options for infections with Enterobacteriaceae producing carbapenem-hydrolyzing enzymes. *Future microbiology*, 2011; **6**(6), 653-666.
15. Collee, J. G., Miles, R. S., and Watt, B. Tests for identification of bacteria. *Mackie and McCartney practical medical microbiology*, 1996; **14,** 131-49.
16. Bauer, A. W. Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*, 1966; **45,** 149-158.
18. Wayne, P. A. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: 20th informational supplement. *CLSI document M100-S20,* 2010.
19. Aghamiri, S., Amirmozafari, N., Fallah Mehrabadi, J., Fouladtan, B., and Samadi Kafil, H. Antibiotic resistance pattern and evaluation of metallo-beta lactamase genes including bla-IMP and bla-VIM types in *Pseudomonas aeruginosa* isolated from patients in Tehran hospitals. *International Scholarly Research Notices*, 2014.
20. Hasan, A. S., Nair, D., Kaur, J., Baweja, G., Deb, M., and Aggarwal, P. Resistance patterns of urinary isolates in a tertiary Indian hospital. *Journal of Ayub Medical College Abbottabad*, 2007; **19**(1), 39-41.
21. Ullah, F., Malik, S., and Ahmed, J. Antibiotic susceptibility pattern and ESBL prevalence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan. *African Journal of Biotechnology*, 2009; **8**(16).
22. Sultana, M., Parvez, A. K., Sultana, K. F., Mukharje, S. K., and Hossain, M. A. Characterization of extended spectrum β-Lactamase producing bactieria isolated from urinary tract infections. *Bangladesh Medical Research Council Bulletin*, 2019; ***45***(1), 23-33.
23. Ahmed, I., Rabbi, M. B., and Sultana, S. Antibiotic resistance in Bangladesh: A systematic review. *International Journal of Infectious Diseases*, 2019; **80**, 54-61.