American Journal of Infectious Diseases

Original Research Paper

Frequency of Carbapenem Resistance among Gram Negative Pathogens in a Tertiary Care Hospital in Southern Pakistan

Waseela Ashraf and Altaf Ahmed

Department of Microbiology, The Indus Hospital, Korangi Crossing Karachi, Pakistan

Article history
Received: 20-05-2015
Revised: 15-07-2015
Accepted: 17-10-2015

Corresponding Author:
Altaf Ahmed
Department of Microbiology,
The Indus Hospital, Korangi
Crossing Karachi, Pakistan
E-mail: altafvirus@yahoo.com

Abstract: Carbapenems are considered the treatment of choice for treating the multi-drug resistance Gram negative bacteria. But resistance to these antibiotics has increased worldwide thus limiting therapeutic options for clinicians. To investigate the frequency and Minimum Inhibitory Concentrations (MICs) of carbapenem resistance in Gram negative organisms at a tertiary care hospital in Southern Pakistan. Seven Hundred and ninety-three carbapenem resistant isolates were identified from different clinical microbiology specimens including blood, urine, pus, wound swabs, tracheal aspirate, catheter and CVP tip. All specimens were processed within 2 h of collection by standard microbiological technique. Antibiotic susceptibility testing was performed by the modified Kirby-Bauer disk diffusion method. Clinical Laboratory Standard Institute (CLSI) was used as reference guide for interpretation of results. MIC testing of imepenem and meropenem was performed on automated Phoenix TM 100. Carbapenem resistance was observed in *Enterobacter cloacae* (77%), *Acinetobacter* spp. (60%), *Pseudomonas* spp. (17.5%), *Klebsiella* spp. (6.1%), *Proteus* spp. (1.6%) and *E. coli* (1.4%) during the year 2010-2014. Trend of resistance observed was 3.2% (51/1150), 3.3% (70/2103), 5.7% (167/2917), 5.8% (172/2950) and 10.0% (333/3329) respectively indicating a rising trend during the study period. MIC testing of imipenem and meropenem was performed by Phoenix TM 100. In 2013, 97 carbapenem resistant isolates were tested for imipenem MIC of which 89 isolates showed MIC >8(µg/mL) while of the 58 isolates tested for meropenem MIC, 54 showed MIC >8(µg/mL) for meropenem. In 2014, 184carbapenem resistant isolates were tested for imipenem MIC and 176 for meropenem MIC of which 155 isolates has MIC >8(µg/mL) for imipenem and 144 isolates has MIC >8(µg/mL) for meropenem. The study shows annual increasing trend of carbepenam resistance in a tertiary care setting in Southern Pakistan thus indicating and identifying serious therapeutic and epidemiological risk of spread of carbapenem resistance. Majority of isolates showed MICs of >8(µg/mL) for both imipenem and meropenemin high frequency. Therefore, continuous monitoring via systemic surveillance studies are necessary to screen resistance in other settings of Pakistan.

Keywords: Gram Negative Bacteria, Carbapenem Resistance, Minimum Inhibitory Concentration, Pakistan

Introduction

Gram negative organisms are involved in causing blood stream, urinary tract, intra-abdominal community acquired and health care associated infections. For treatment, broad spectrum Beta-lactams aminoglycosides, cephalosporins and flouroquinolones are used as major anti-microbial agents (Agarwal *et al*., 2006). However, largenumber of Gram negative bacteria is capable of producing beta lactamases and Extended Spectrum Beta Lactamases (ESBLs). These enzymes confer resistance to the respective bacteria to various classes of beta lactam antibiotics. To counter this, Carbapenem antibiotics have been developed. These

antibiotics are stable to Amp C beta lactams and extended spectrum beta lactamases and capable of eradicating beta lactamases producing bacteria (Swenson *et al*., 2006).

Antimicrobial resistance leads to undesirable outcomes including increased mortality, hospital stay and costs. In addition delay in institution of effective therapy, lesser definitive therapy and greater virulence of some strains are responsible for antimicrobial resistance (Lledo *et al*., 2009; Lolans *et al*., 2006). Furthermore therapeutic options are not yet available and viable effective option for the treatment of invasive blood stream infections is very limited.

Over the past decade, a large number of studies have documented emergence of resistance, frequently known as Carbapenamases among Gram negative bacteria. Carbapenamases are a class of enzymes that can efficiently hydrolyze most beta lactams including Carbapenems (Paterson and Bonomo, 2005). Emerging types of carbapenamases include *Klebsiella Pneumoniae* Carbapenemase (KPC), Verona integron-encoded metallo-β-lactamase (VIM), Oxacillinase-48 (OXA-48) and New Delhi metallo-β-lactamase-1 (NDM). Most of the transposable enzymes against carbapenems producing encoding genes are carried on integrons as cassettes which aid their rapid spread among organisms and confer resistance to both beta lactam and other antimicrobial agents. Carbapenem resistance may be mediated by porin loss and hyper expression of efflux pumps (Peleg *et al*., 2008). Resistance enzymes encoding genes have been detected commonly in *Enterobactericeae*, *Pseudomonas* spp. and *Acinetobacter* spp (Tsakris *et al*., 2000).

From Pakistan, limited studies on carbapenam resistance have been documented. Therefore, the aim of the study was to determine the frequency of carbapenem resistance in Gram negative organisms in a tertiary care hospital setting so that evidence on the prevalence of carbapenem resistance from southern Pakistan can be documented.

Materials and Methods

This study was performed at the Indus Hospital (TIH) Karachi. TIH is the 150 bedded tertiary care hospital with large influx of patients from low socioeconomic background. It has a fully computerized medical record system and all lab results are directly entered into the system. Furthermore, the TIH microbiology lab follows international standards for isolation as well as drug susceptibility testing.

Briefly, specimens including pus, wound swabs, tracheal aspirates, catheter, CVP tips, sputum, body fluids and tissue were processed on blood culture within 2 h of collection by standard microbiological technique. Blood culture positive samples were inoculated on blood, chocolate and MacConkey agar. Urine specimens were inoculated on Cystien Lactose Electrolyte Deficient (CLED) agar and incubated at 35°C for 18-24 h in aerobic atmosphere. Organisms were identified on the basis of standard microbiological techniques (Cheesbrough, 2000; Dortet *et al*., 2006)**.**

By following Clinical Laboratory Standards Institute (CLSI) Guidelines, antimicrobial susceptibility testing was performed by the modified Kirby-Bauer disk diffusion method. In Carbapenem group disk diffusion testing was performed on meropenem and imipenem. MICs testing of meropenem and imipenem was performed on Phoenix automation.

All data was retrospectively collected via the medical record system. The records showed that a total of 12,849 Gram negative isolates were isolated from different clinical specimens during the year 2010-2014. Medical records indicated that automated MICs for isolates were performed in 2013-14 only due to non-availability of Phoenix system in the lab during 2010-2012. Accordingly, approximately 97 and 58 isolates were tested for imipenem and meropenem MIC in 2013 while 184 and 176 isolates were tested for imipenem and meropenem MIC in 2014. Furthermore, control strain of *Pseudomonas aeruginosa* ATCC 27853 was used for the quality control of both disc diffusion and MIC methods.

Results

Out of 12849 isolates, 793 isolates (6.2%) were found to be resistant to both meropenem and imipenem. The yearly frequency of carbapenem resistant organisms is given in Table 1. While the trend of carbapenem resistance from 2010-14 is 3.2% (51/1150), 3.3% (70/2103), 5.7% (167/2917), 5.8% (172/2950) and 10.0% (333/3329) respectively.

In 2013, 97 isolates were tested for imipenem MIC testing. Of these isolates, in *Enterobacter cloacae* 100% (3/3), in *Pseudomonas* spp. 100% (20/20) in *Acintobacter*spp 97.5% (19/20), *E. coli* 82.3% (14/ 17) and in *Klebsiella* spp. 72.2% (13/18) had MIC value >8 µg/mL. Furthermore, 58 isolates were tested for meropenem MIC testing. Of these 54 isolates had MIC >8 µg/mL. In *Pseudomonas* spp. 100% (8/8), in *Acinetobacter* spp. 100% (32/32), in *Klebsiellasp p*85.7% (6/7) and in *E. coli*, 70% (7/10) isolates had MIC >8 µg/mL. In *Enterobacter cloacae* only a single isolate was processed which also had MIC at breakpoint >8µg/mL.

Table 1. Frequency of carbapenem resistant organisms from year 2010-2014

Organism	Total no.	Carbapenem resistant % (n)
Enterobacter cloacae	22	77 (17)
Acinetobacter spp.	450	60 (271)
Pseudomonas spp.	1420	17.5 (249)
Klebsiella spp.	2147	6.1 (132)
Proteus spp.	835	1.6 (13)
E. coli	7975	1.4 (111)

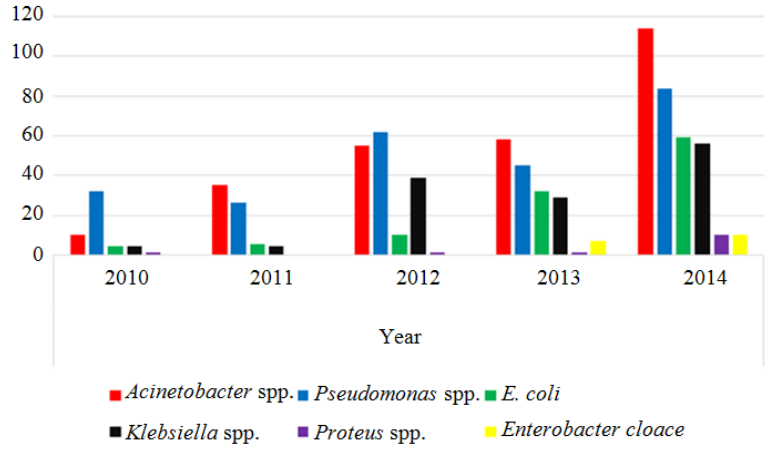

Fig. 1. Organisms showing trend of Carbapenem resistance

In 2014, 184 isolates were tested for imipenem MIC testing. Of these, in *Acintobacter* spp. 100% (72/72), in *Pseudomonas* spp. 87.5% (35/40), *E. coli* 80% (28/35), in *Klebsiella* spp. 59.2% (16/27) and in *Enterobacter cloacae*, 40% (4/10) isolates had MIC>8 µg/mL. Furthermore, 176 isolates were tested for meropenem MIC testing. In *Acinetobacter* spp. 98.6% (72/73) *E. coli,* 80% (24/30), in *Pseudomonas* spp. 76.9% (30/39), in *Klebsiella* spp.70.8% (17/24), in *Enterobacter cloacae*, 30% (3/10) and in isolates had MIC>8 µg/mL.

Discussion

Carbapenem resistant organisms are co resistant to almost all classes of antibiotics (Marchaim *et al*., 2007). Acquired carbapenemases are a large group of beta lactamases of high structural diversity posing a significant health problem due to high morbidity and mortality (Struelens *et al*., 2010; Sengstock *et al*., 2010). Resistance mechanisms to Carbapenem are frequently found on mobile genetic elements that possess the potential to spread widely (Gupta *et al*., 2011).

The first Carbpenem producing strain was isolated in Japan in 1991 and reported in India in 2002 (Yong *et al*., 2002; Navaneeth *et al*., 2002; Renu *et al*., 2010). According to the 2009 data from the European Antimicrobial Resistance Surveillance network (EARS-net) rate of carbapenem resistance among invasive *Klebsiella pneumoniae* infections were 43.5% in Greece, 17.0% in Cyprus, 1.3% in Italy, 1.2% in Belgium and below 1% in other 23 reporting countries (Evans, 2014). From Pakistan, a study conducted at Army Medical College Rawalpindi in 2010 reported resistance to carbapenems in both *Acinetobacter baumanni* and *Pseudomonas aeruginosa* (Kaleem *et al*., 2010). In our study setting, we have also observed rising trend of carbapenem resistance in Gram negative organisms during the study period. Alarmingly, the MICs for most organisms were found to be >8 µg/mL demonstrating that carbapenem antibiotics have limited usefulness in treating beta lactamase producing isolates.

Carbapenems are effective therapeutic agents against highly resistant pathogens such as *Pseudomonas* spp. and *Acinetobacter* spp. Spread of this resistance among bacteria would seriously restrict therapeutic options. This challenging situation is difficult to manage in a resource limited country. On the contrary, the situation continues to become more difficult by the arbitrary use of antibiotics in the population. The incidence of carbapenem resistance in hospital setting of Southern Pakistan, serves as an alarm for infection control management and pinpoints the serious therapeutic and epidemiological risk of the spread of carbapenem resistance in other hospital settings as well. Early detection and infection control practices are the best defense against these organisms therefore continuous systemic surveillance studies are necessary to screen carbapenem resistant isolates. To prevent the spread of these organisms and to save the therapeutic options judicious use of carbapenem is essential. Health care providers must be aware of the importance of carbapenem resistance to prevent in the possibility of out breaks in health care institutions.

Conclusion

This study provides useful data on the current status of carbepenam resistance in health care settings of Pakistan. Findings from this study can be used as a baseline for conducting further studies on this aspect from other hospital settings in Southern Pakistan.

Acknowledgment

We would like to thank the TIH microbiology and administrative staff for their support. We would also like to thank Dr. Afsheen Raza for her guidance during manuscript preparation and review.

Author Contributions

Waseela Ashraf: Collected and analyzed the data as well as prepared the manuscript.

Altaf Ahmed: Planned and designed the study and reviewed the final draft of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Ethics

The study was conducted after approval from institutional review board.

References

Agarwal, V.A., S.A. Dongre and R.M. Powar, 2006. Antimicrobial resistance profile of Pseudomonas aeruginosa producing metallo-beta-lactamases. Indian J. Med. Res., 124: 588-588.

Swenson, J., J. Patel, J. Jorgensen, P. Murray and E. Baron *et al*., 2006. Special phenotypic methods for detecting antibacterial resistance. Manual Clinical Microbiol., 1: 1173-92.

Lledo, W., M. Hernandez, E. Lopez, O. Molinari and R. Soto *et al*., 2009. Guidance for control of infections with carbapenem-resistant or carbapenemase-producing Enterobacteriaceae in acute care facilities. Morbidity Mortality Weekly Report, 58: 256-8.

Lolans, K., T.W. Rice, L.S. Munoz-Price and J.P. Quinn, 2006. Multicity outbreak of carbapenem-resistant Acinetobacter baumannii isolates producing the carbapenemase OXA-40. Antimicrobial Agents Chemotherapy, 50: 2941-5. DOI: 10.1128/AAC.00116-06

Paterson, D.L. and R.A. Bonomo, 2005. Extended-spectrum β-lactamases: A clinical update. Clin. Microbiol. Rev., 18: 657-86. DOI: 10.1128/CMR.18.4.657-686.2005

Peleg, A.Y., H. Seifert and D.L. Paterson, 2008. Acinetobacter baumannii: Emergence of a successful pathogen. Clin. Microbiol. Rev., 21: 538-582. DOI: 10.1128/CMR.00058-07

Tsakris, A., S. Pournaras, N. Woodford, M.F. Palepou and G.S. Babini *et al*., 2000. Outbreak of infections caused by Pseudomonas aeruginosa producing VIM-1 carbapenemase in Greece. J. Clin. Microbiol., 38: 1290-1292.

Cheesbrough, M., 2000. Summary of the clinical and laboratory features of microorganism. District laboratory practice in tropical countries (Part 2). Cambridge University Press.

Dortet, L., P. Legrand, C.J. Soussy and V. Cattoir, 2006. Bacterial identification, clinical significance and antimicrobial susceptibilities of Acinetobacter ursingii and *Acinetobacter* schindleri, two frequently misidentified opportunistic pathogens. J. Clinical Microbiol., 44: 4471-8. DOI: 10.1128/JCM.01535-06

Marchaim, D., S. Navon-Venezia, A. Leavitt, I. Chmelnitsky and M.J. Schwaber *et al*., 2007. Molecular and epidemiologic study of polyclonal outbreaks of multidrug-resistant *Acinetobacter* baumannii infection in an Israeli hospital. Infect. Control Hosp. Epidemiol., 28: 945-950. DOI: 10.1086/518970

Struelens, M., D. Monnet, A. Magiorakos, O. Santos and F. Connor *et al*., 2010. European NDM-1 survey participants. New Delhi metallo-beta-lactamase 1-producing Enterobacteriaceae: Emergence and response in Europe. Euro Surveill., 15: 19716.

Sengstock, D., R. Thyagarajan, J. Apalara, A. Mira and T. Chopra *et al*., 2010. Multidrug-resistant *Acinetobacter* baumannii: An emerging pathogen among older adults in community hospitals and nursing homes. Clin. Infect. Dis., 50: 1611-6. DOI: 10.1086/652759

Gupta, N., B.M. Limbago, J.B. Patel and A.J. Kallen, 2011. Carbapenem-resistant Enterobacteriaceae: Epidemiology and prevention. Clin. Infect. Dis., 53: 60-67. DOI: 10.1093/cid/cir202

Yong, D., K. Lee, J.H. Yum, H.B. Shin and G.M. Rossolini *et al*., 2002. Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J. Clinical Microbiol., 40: 3798-801. DOI: 10.1128/JCM.40.10.3798-3801.2002

Navaneeth, B., D. Sridaran, D. Sahay and M. Belwadi, 2002. A preliminary study on metallo-beta-lactamase producing *Pseudomonas* aeruginosa in hospitalized patients. Indian J. Med. Res., 116: 264-267.

Renu, G., T. Rajeev and S. Smita, 2010. The existence of metallo beta lactamases in carbapenem susceptible Gram negative bacilli: A cause for concern. J. Clin. Diagn. Res., 4: 2679-84.

Evans, R., 2014. European centre for disease prevention and control. Nursing Standard, 29: 30-30.

Kaleem, F., J. Usman, A. Hassan and A. Khan, 2010. Frequency and susceptibility pattern of metallo-beta-lactamase producers in a hospital in Pakistan. J. Infect. Dev. Countrie., 4: 810-813. PMID: 21252461