MEDICAL CHANNEL

Original Article

EMERGENCE OF IMIPENEM RESISTANCE IN GRAM NEGATIVE BACTERIA

** Muhammad Salman Rasool, ** Fraz Siddiqui, *** Munazza Ajaz, **** Sheikh Ajaz Rasool, ***** Saleem Hafiz,*

* Department of Microbiology, University of Karachi, Pakistan ** Fatima Bai Hospital Karachi Pakistan *** Federal University of Science and Technology, Karachi, Pakistan **** Department of Microbiology, University of Karachi, Pakistan

ABSTRACT

Emergence of imipenem (IPM) resistance in Gram negative rods has become a global healthcare problem. In Karachi there has been a steady increase in Multidrug resistant isolates of Gram negative organisms and also imipenem resistance is emerging. Multidrug resistant (MDR) Gram negative rods (GNR) were isolated and identified from various clinical specimens (n=29669) e.g respiratory, blood, urine, wound, pus and fluids, by using various culture media and automated profile index (API) system. They were subjected to antibiotic screening by Kirby-Bauer disk diffusion method according to CLSI guidelines (2012-2014). *E.coli* (48%), *Klebsiella pneumoniae* (19%), *Pseudomonas aeruginosa* (11%), *Acinetobacter baumanni* (5%) and other GNR (13%) were found to be multidrug resistant (MDR) along with imipenem resistance. Out of total number of GNR (56%) were sensitive to most groups of antibiotics, Resistance to imipenem was 44%. Imipenem resistance and multidrug resistance are increasing in our country because of misuse of antibiotics i.e. self medication and injudicious use of antibiotics and inappropriate antibiotic therapy. If this practice is not controlled even the life saving drugs would be ineffective and super bugs would develop.

INTRODUCTION

Multi drug resistant (MDR) gram negative rods including imipenem resistance spread like untreated cancer and is becoming a global problem for healthcare professionals (Maviglia *et al*., 2009; Mushi *et al*., 2014). are major source of morbidity and mortality and cost in many clinical settings (Naiemi *et al*, 2006; Waikhom and Devi, 2012). MDR gram negative rods are usually resistant to atleast three groups of antibiotics, these bacteria presently create a very serious problem for antibiotic treatment especially in critically ill patients admitted in intensive care units (Chakraborty *et al*.,

Corresponding Author:
MUHAMMAD SALMAN RASOOL
Department of Microbiology, University of Karachi, Pakistan

2011). Carbapenem antibiotics such as imipenem, has been used as last resort for treatment of infections caused by multidrug resistant bacteria. Resistance to this antibiotic in multidrug resistant gram negative rods becomes a great threat for survival of patients infected by such superbugs (Mattner, 2012).

The mechanisms of resistance could be due to enzymes production, permeability factors and mutations which may result by miss use of antibiotics. Enzyme associated antibiotic resistance in Gram negative rods (GNR) may include Extended spectrum betalactamases (ESBLs), AmpC cephalosporinases and Carbapenemases (Sharma *et al*., 2013; Motayo *et al*., 2013). Permeability factors include change in drugs receptor or porins located at cell envelope and cell membrane, biofilm formation and efflux pumps (Lupo *et al*., 2012). Mutations could be induced or spontaneous which may result due to physical and chemical mutagenic agents. Misuse of antibiotic is mainly the consequence of self medication and inappropriate antibiotic therapy (Van dar Horst *et al* 2011). All these factors may contribute to the emergence of MDR and Imipenem resistance.

MATERIALS AND METHODS

Isolation, identification and cultural characteristics: Twenty Thousand seven Hundred and twnty eight Gram negative isolates from various clinical samples were collected from different parts of Karachi and identified on the basis of conventional biochemical reactions on various media including, Citrate agar, Triple sugar iron (TSI) agar, Urea agar and Sulphide indole motility (SIM) medium and by automated profile index (API) system (Koneman *et al*, 2006) and wherever needed API was used.

Antibiotic susceptibility testing:

Antibiotic sensitivity testing was performed according to Clinical laboratory standard Institute (CLSI, 2012). Amikacin(AK30**µ**g), Ampicillin (AMP10**µ**g), Amoxicillin-clavulanic acid (AMC20/10**µ**g), Aztreonam (ATM30**µ**g), Cefipime (FEP30**µ**g), Cefixime (CFM5**µ**g), Ceftriaxone (CRO30**µ**g), Cefoperazone-sulbactam (SCF105**µ**g), Cefotaxime (CTX30**µ**g), Ciprofloxacin (CIP5**µ**g), Imepenem (IPM10**µ**g), Nalidixic acid (NA30**µ**g), Nitrofurantoin (F300**µ**g), Pipracillin-tazobactam (TZP100/10**µ**g), Polymyxin B (PB300**µ**g) and Trimethoprim-sulfamethoxazole (SXT1.25/ 23.75**µ**g) discs were used. The standard abbreviations used for the antibiotics have been quoted throughout the paper. All the antibiotic discs were obtained from (Oxoid). MacFarland 0.5 suspension of the isolate was made in normal saline that was spread by swab over the Muller Hinton (MH) agar (Oxoid) and appropriate discs of the above indicated antibiotics were placed at the 15 mm distance from each other (CLSI 2012). Quality control strains *E.coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used for the standardization of antibiotic sensitivity testing.

RESULTS

Figure 1 gives the breakdown of gram negative isolates collected from various clinical samples.

Figure 2 gives the breakdown of various Gram negative isolates collected including *E.coli* (48%), *Klesiella pneumoniae* (19%), *Pseudomonas aeruginosa* (11%),

FIGURE-1
OVER ALL SPECIMENS COLLECTED

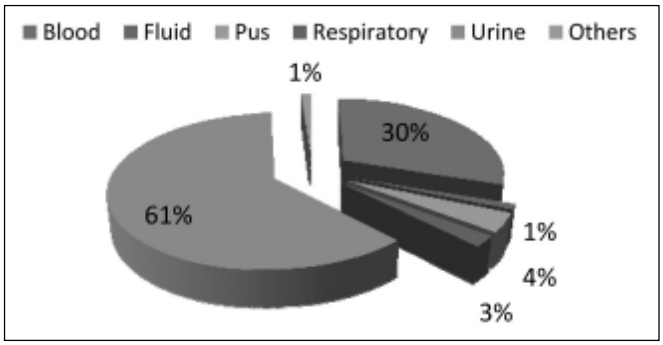


FIGURE-2
A VARIETY OF GRAM NEGATIVE RODS (20728)

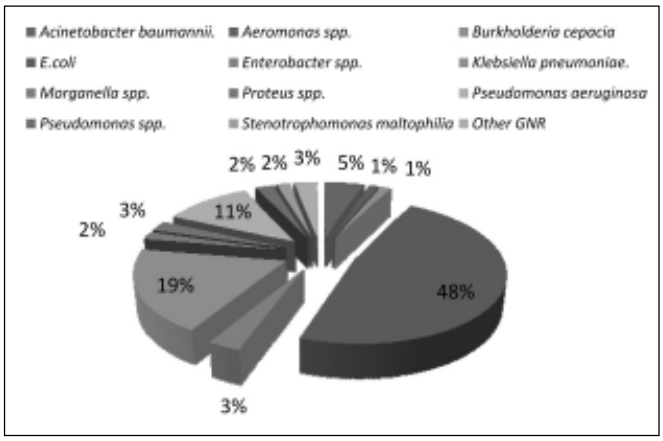
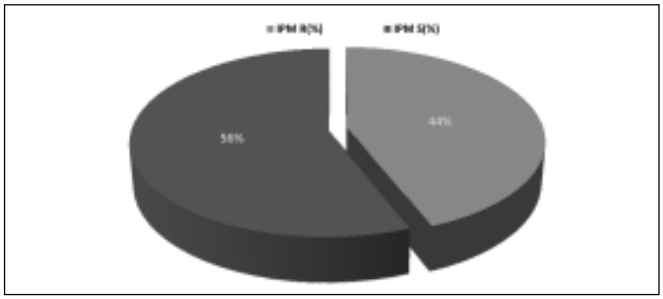


FIGURE-3
ANTIBIOTIC SENSITIVITY SUMMARY OF GNR



Acinetobacter baumanni. (5%), *Proteus* spp. (3%), *Enterobacter* ssp. (3%), *Morganella* spp. (2%), *Pseudomonas* spp.(2%), *Stenotrophomonas maltophilia* (2%), *Aeromonas* spp. (1%), *Burkholderia cepacia* (1%) and other GNR (3%).

Out of total number of GNR 56% were found to be sensitive to most groups of antibiotics, 44% were MDRincluding Imipenem resistance (fig-3).

Keys: IPM R(%)=Percentage of Imipenem resistant Gram negative bacilli, IPM S (%)=Percentage of Imipenem sensitive Gram negative bacilli, IPM(R)+MDR(%)=Percentage of Multidrug resistant gram negative bacilli, showing Imipenem resistance, MDR(%)=Percentage of Multidrug resistant Gram negative bacilli.

DISCUSSION AND CONCLUSION

Imipenem resistance and multidrug resistance among Gram negative bacteria are on the increase this poses a great threat to the community in our country because of misuse of antibiotics i.e. self medication and injudicious use of antibiotics and inappropriate antibiotic therapy. If this practice is not controlled soon a very good drugs would become useless. So there is a need to identify novel antimicrobial agents, new targets and clinical microbiology laboratories must develop the guidelines to detect the mechanisms of antibiotic resistance.In Taiwan a study by Shao-Xing Dong et. al found meropenem, doripenem and imipenem to be equally efficacious

REFERENCES

1. Chakraborty D, Basu S and Das S (2011). Study on some gram negative multi drug resistant bacteria and their molecular characterization. Asian J Pharm Clin Res, 4(1): 108-112.
2. CLSI (2012). Performance Standards for Antimicrobial Susceptibility Testing, 22nd Informational Supplement. M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute.
3. Dong SX, Wang JT, Chang SC. Activities of doripenem against nosocomial bacteremic drug-resistant Gram-negative bacteria in a medical center in Taiwan. J Microbiol Immunol Infect 2012; 45(6):459-64.
4. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC jr (2006). Guidelines for the collection , transport, processing, analysis and reporting of cultures from specific specimen sources. In: Color Atlas and Textbook of Diagnostic Microbiology 6th edition Lipincott Williams and Wilkins, 68-105.
5. Lupo A, Coyne S and BerendonkTU (2012). Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Frontiers in Microbiology. 3(18): 1-13.
6. Mattner F, Bange FC, Meyer E, Seifert H, Wichelhaus TA, and Chaberny IF (2012). Preventing the Spread of Multidrug-Resistant Gram-Negative Pathogens. Dtsch Arztebl Int, 109(3): 39–45.
7. Maviglia R, Nestorini R, and Pennisi M (2009). Role of old antibiotics in multidrug resistant bacterial infections. Curr Drug Targets. 10:895–905.
8. Motayo BO, Akinduti PA, Adeyakinu FA, Okerentugba PO, Nwanze JC, Onoh CC, Innocent-Adiele HC and Okonko IO (2013). Antibiogram and plasmid profiling of carbapenemase and extended spectrum ?-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae in Abeokuta, South western, Nigeria. Africa Health Sciences, 13(4): 1140 – 1146.
9. Mushi MF, Mshana SE, Imirzalioglu C, and Bwanga F (2014). Carbapenemase Genes among Multidrug Resistant Gram Negative Clinical Isolates from a Tertiary Hospital in Mwanza, Tanzania. BioMed Research International, 2014:1-6.
10. Naiemi NA, Heddema ER, Bart A, Jonge ED,Vandenbroucke-Grauls CM, Savelkoul PH and Duim B (2006). Emergence of multidrug-resistant Gram-negative bacteria during selective decontamination of the digestive tract on an intensive care unit. Journal of Antimicrobial Chemotherapy, 58: 853–856.
11. Sharma M, Pathak S and Srivastava P (2013). Prevalence and antibiogram of Extended Spectrum ?-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing Escherichia coli and Klebsiella spp. Journal of Clinical and Diagnostic Research, 7(10): 2173-2177.
12. Van dar Horst MA, Schuurmans JM, Smid MC, Koenders BB and ter Kuile BH (2011). De Novo acquisition of resistance to three antibiotics by Escherichia coli. Microbial Drug Resistance, 17:141-147.
13. Waikhom KD and Devi KS (2012). Emergence of multidrug resistant bacterial infection in HIV/AIDS cases. theHealth, 3(2): 49-52.
14. Mustafa M, Chan WM, Lee C, Harijanto E, Loo CM, Van Kinh N, et al. A PROspective study on the Usage patterns of Doripenem in the Asia-Pacific region (PROUD study). Int J Antimicrob Agents 2014; 43(4):353-60.