ANTIMICROBIAL ACTIVITY OF ERYTHROMYCIN AND CLARITHROMYCIN AGAINST CLINICAL ISOLATES OF ESCHERICHIA *COLI*, STAPHYLOCOCCUS *AUREUS*, KLEBSIELLA AND PROTEUS BY DISC DIFFUSION METHOD

NAVEED WALI KHAN, FOUZIA HASSAN, BAQAR S NAQVI AND SYED MUHAMMAD FARID HASAN*

Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan

ABSTRACT

Fifty clinical isolates comprising of Escherichia *col*i, Staphylococcus *aureus*, Klebsiella and Proteus were collected from different local pathological laboratories and their resistant pattern against two well known macrolides; erythromycin and clarithromycin were studied using disc diffusion method. Klebsiella (41.67% against erythromycin and 58.34% against clarithromycin) and Proteus (66.67% against erythromycin and clarithromycin) species were found to be more resistant against the studied macrolides as compared to the rest of organisms. In case of Staphylococcus *aureus* and Escherichia.*coli*, resistant found were 27.78% and 23.54% against erythromycin and 22.23% and 35.30% against clarithromycin respectively. It is concluded from these figures that microbial resistance against these macrolides are increasing in our population which is alarming and therefore it is recommended to physicians to prescribe these antibiotics unless no other substitute is available in clinical practices.

Keywords: *Escherichia coli, Staphylococcus aureus, Klebsiella, Proteus,* macrolides.

INTRODUCTION

Antimicrobial resistance is an increasing problem that contributes to morbidity, mortality and increased health care cost (Gums 2002) with tremendous variability not only amongst pathogens causing various clinical infections in different geographic regions, but also over time in specific areas (Hsueh *et al.,* 2010). Asia Pacific region has been the area with the highest levels of antimicrobial resistance amongst the five global regions studied (Hawser *et al.,* 2009). Resistance means that an organism ceases to be killed or inhibited by a drug (Duerden *et al.,* 1998). Human misuse of antibiotics plays a major role in resistance. This problem may occur when antibiotics are used in every disease. It is a common practice that many patients discontinue antibiotic therapy as soon as they feel better irrespective of the outcomes. These aborted treatments encourage drug resistance (Lewis 1995). Another source of resistance against antibiotics is animals. To prevent diseases and to keep animals well, drugs are given to them and later they are slaughtered for food. In addition, sometimes antibiotics are administered at low levels in feed for long durations and the purpose besides other reasons is to increase the rate of weight gain. It should be noteworthy that antibiotic does not technically cause resistance, but allows it to happen by creating a situation where an already existing variant can flourish (Lewis, 1995).

Although medical practices are flourishing very fast in this era, yet many diseases are there that needs suitable agents to get cured. Due to growing resistance, many bacterial infections even today are not being treated effectively. If these infections are not treated properly then they may become fatal threat in the future (Paustian 1999).

Among the antimicrobial agents, macrolides group is widely prescribed to treat various diseases. These are safe antibiotics and are useful for those patients that are hypersensitive to penicillins and other beta lactams (Strahilevitz and Hooper 2010). Like other antibiotics, resistance by organisms against this group has also been reported by many workers in other parts of the world. Resistant pathogens are associated with higher morbidity and mortality than those caused by susceptible pathogens therefore, the global impact of increasing resistance is a major concern (Isturiz 2008). Enterobacteriaceae, most notably *Escherichia coli* and *Klebsiella pneumoniae*, are among the most important causes of serious nosocomial and community-associated bacterial infections in people, and resistance of these bacteria to antimicrobial drugs is a serious concern (Pitout 2010).

The objective of the present work was to determine the resistance pattern of fifty clinical isolates comprising of Escherichia *coli*, Staphylococcus *aureus*, Klebsiella and Proteus against two representative members of macrolide group namely erythromycin and clarithromycin which were selected for their wider use by disc diffusion method.

MATERIALS AND METHODS

Equipment and apparatus

Autoclave (Model LS-2, Taiwan), Hot air oven (YCo-NO1, Gemmy, Taiwan), Incubator (B-5028 Heraeus,

***Corresponding author:** e-mail: smfhassan@uok.edu.pk

Bremen, Germany), Balance (Model-2842, Sartorius, Germany) Mueller Hinton Agar (Merck), Mueller Hinton Broth (Merck), glassware (Pyrex, England)

Collection of clinical isolates

Clinical isolates were collected from different pathological laboratories located in Karachi. They are identified as *Escherichia coli* (17), *Staphylococcus aureus* (18), *Klebsiella* (12) and *Proteus* (3).

Antimicrobial agents

Standard discs of Erythromycin and Clarithromycin were purchased from Oxoid, UK.

Preparation of media

Mueller Hinton Agar and Mueller Hinton Broth were prepared and sterilized according to manufacturers instructions (Merck).

Preparation of media plates

Mueller Hinton Agar was poured into sterile Petri dish about 20-25 ml per plate. The plates were then aside on a flat surface and allowed to solidify for 15 minutes.

Preparation of Inoculum

The inoculation was prepared by touching the top of the colonies of the isolates with sterile wire loop and suspending in a tube containing 2-3 ml of broth. All work was carried out near flame. The tubes are then incubated at 37°C for few hours.

Inoculation of plates

A sterile swab was used for this purpose. Sterile swab was dipped into a broth suspension of organism. Excess fluid was removed by pressing and rotating the swab against the side of tube above the level of suspension. The swab was then steak evenly over the surface of the medium in three directions, rotating the plates approximately 60 degree to ensure even distribution. After inoculation, surface of agar was allowed to dry.

Placement of antibiotic disc

By using sterile forcep, the appropriate antimicrobial discs of erythromycin and clarithromycin were placed on the agar surface one by one side by side. Each disc was slightly pressed down to ensure its contacts with agar.

Incubation of Plates

Within 30 minutes of applying discs, the plates were inverted and incubated at 37°C for 24 hours.

Examination of Plates

After 24 hours of incubation, the plates were examined and zone of inhibition was measured.

RESULTS

In the present study, resistant pattern of fifty (50) clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* and Proteus were studied using erythromycin and clarithromycin and the results are depicted in figs. 1 and 2 respectively. The results revealed that 23.54% clinical isolates of *Escherichia coli*, 27.78% *Staphylococcus aureus*, 41.67% *Klebsiella* and 66.67% Proteus were resistant to erythromycin. In case of clarithromycin, the results showed that 35.30% clinical isolates of *Escherichia coli*, 22.23% *Staphylococcus aureus*, 58.34% *Klebsiella* and 66.67% Proteus were resistant to clarithromycin. From these figures, it is clear

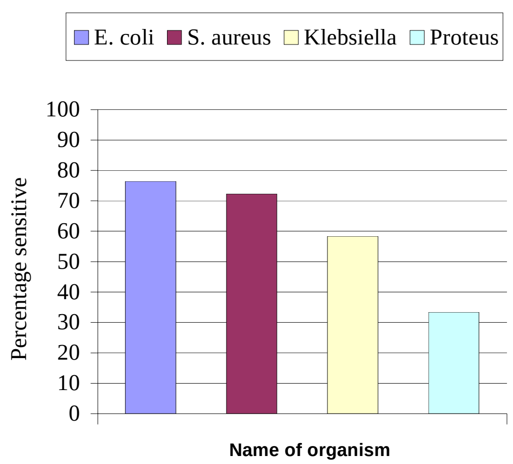

Fig. 1: Percentage of organisms sensitive to Erythromycin

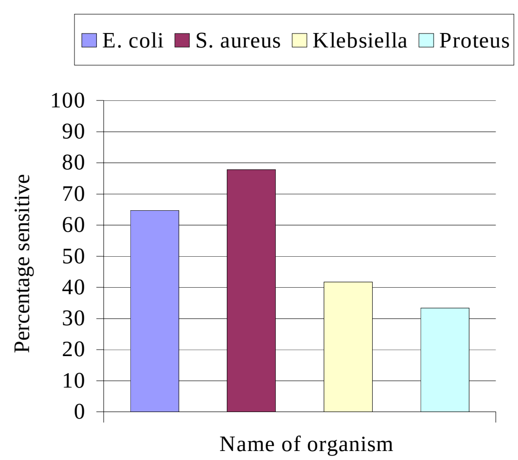

Fig. 2: Percentage of organisms sensitive to Clarithromycin

that *E. coli* and *Klebsiella* are more resistant against clarithromycin as compare to erythromycin but in case of *S. aureus*, it appears that erythromycin is acquiring more resistance as compare to its counterpart. However, in case of Proteus very high as compare to the rest of clinical isolates, yet a similar resistant pattern were observed against these macrolides.

DISCUSSION

Major factor limiting the long-term use of antimicrobial agents is resistance. Before antibiotics era, many people died of bacterial infections caused by pathogens as Staphylococcus *aureus*, Streptococcus pyrogenes, and Streptococcus pneumonia. Use, abuse or misuse of antimicrobial agents has encouraged the evolution of bacteria towards resistance that often results in therapeutic failure (Straut *et al.,* 1995). Prescribing practice of specific class of antibiotics to certain organisms has been found to play a critical role in development of resistance against that antibiotic (Metz-Gercek *et al.,* 2009; Costelloe *et al.,* 2010). Thus, anitmicrobial resistance findings and understanding are necessary to help minimize the emergence of multidrug-resistant organisms by promoting prudent use of antibiotics (Anderson and Kaye 2009), for this purpose, the need for general public to be appropriately informed on use of antibiotics has been emphasized (Eurosurveillance editorial team 2010).

Resistance to erythromycin is becoming a serious clinical problem (Mycek *et al.,* 2000). Clarithromycin show cross-resistance with erythromycin (Finkel *et al.,* 2009). A study on Prevalence of antimicrobial resistance among gram-negative isolates in an adult intensive care unit at a tertiary care center shows decreased susceptibility of *E. coli* and *Klebsiella*, besides other organisms, to various antibiotics (Al-Johani *et al.,* 2010). *E. coli* has recently represented highest prevalence of resistance against ampicillin and trimethoprim-sulfamethoxazole (Araújo *et al.,* 2010). A recent data shows that hospital-acquired isolates of *K. pneumoniae*, rather than outpatient isolates, are more likely to be resistant to multiple antibiotics (Al-Tawfiq and Antony, 2007).

Several workers throughout the world has reported resistance of various organisms against erythromycin and clarithromycin. Malhotra-Kumar *et al* in 2007 studied the direct effect of antibiotic (azithromycin and clarithromycin) exposure on resistance in the oral streptococcal flora of healthy volunteers using double blind design. In this study, volunteers were treated with azithromycin, clarithromycin or placebo and pharyngeal swabs were obtained according to the protocol (180days) and were assessed for macrolides resistant. Both macrolides significantly increased the proportion of macrolide-resistant streptococci compared with the placebo at all points studied. This study shows that macrolide use is the single most important factor of the emergence of macrolide resistance in *vivo* and the physicians prescribing these drugs should take into consideration the side-effects of such antibiotics (Malhotra-Kumar *et al.,* 2007).

In another study, erythromycin resistance in Campylobacter *coli* from meat animals were investigated and it was found that it frequently encountered and could represent a substantial barrier to antibiotic treatment of human infections (Kim *et al.,* 2006). In another work, resistance rates to three antimicrobials (ciprofloxacin, erythromycin and naldixic acid) in Camylobacter isolated from organicaly and intensively reared chickens in London were taken into consideration. Using preset breakpoints, all isolates from all groups of chickens were identified as resistant to erythromycin (Soothornchaikul *et al.,* 2006). Similarly in another research conducted by Yague-Guiraro *et al.,* 2005, the activity of seven macrolides, clindamycin and telithromycin against clinical isolates of Corneybacterium species were studied and a high resistance were found against macrolides (Yague-Guiraro *et al.,* 2005). In a long term study (January 1999 and December 2002), Grivera *et al.,* 2006 recovered 1577 isolates of S. pyrogenes from children with tonsillopharyngitus living in various areas of western Greece. Erythromycin resistance was observed in 379 (24%) of the isolates (Grivera *et al.,* 2006). Antimicrobial susceptibility of Staphylococcus *aureus* and viridians group streptococci strains collected from the forearm and saliva of 30 patients at high risk of endocarditis were studied by Groppo *et al.,* 2005. Of the Staphylocccus strains, 50% were resistant to ampicillin, 53.3% to amoxicillin, 60% to penicillin G, 13.3% to amoxicillin/clavulanate, 20% to azithromycin, 27.6% to clarithromycin, 23.3% to erythromycin, 3.3% to cefazolin and 6.7% to clindamycin. Regarding streptococci, 16.7% of the strains were resistant to ampicillin, 16.7% to amoxicillin, 23.3% to azithromycin, 23.3% to clarithromycin, 30% to erythromycin, 13.3% to cefazolin, 26.7% to clidamycin, 16.7% to penicillin G and 3.3% to amoxicillin/clavulanate (Groppo *et al.,* 2005). Drago *et al.,* 2005 compared in *vitro* antimicrobial activity of several antibiotics against 287 strains of S. pyogenes by the broth microdilution method. Ceftibuten and Cefaclor showed the best antimicrobial activity while MIC values for telithromycin were higher against constitutively MLS resistant strains rather than against the other phenotypes (Drago *et al.,* 2005). We in the present work also found resistance against macrolides and thus our study corroborates with the previous studies.

CONCLUSION

In our population, organisms are developing resistance against macrolides. We recommend physicians to prescribe this group unless no other alternative is available. Also, prescribe that antimicrobial agent which is most effective in particular circumstances, otherwise it seems that treatment of some infectious diseases may become impossible in the near future.

ACKNOWLEDEGMENT

The authors are grateful to M/s. Abbott Laboratories Pakistan Ltd. for providing financial support to carry out this work and Mr. Yasir Faraz Abbasi, College of Pharmacy, Ziauddin University, Karachi for his excellent assistance in the completion of this manuscript.

REFERENCES

Al Johani SM, Akhter J, Balkhy H, El-Saed A, Younan M and Memish Z (2010). Prevalence of antimicrobial resistance among gram-negative isolates in an adult intensive care unit at a tertiary care center in Saudi Arabia. *Ann. Saudi Med*., 30(5): 364-369.

Al-Tawfiq JA and Antony A (2007). Antimicrobial resistance of Klebsiella pneumoniae in a Saudi Arabian hospital: results of a 6-year surveillance study, 1998-2003. *J. Infect. Chemother*., 13(4): 230-234.

Anderson DJ and Kaye KS (2009). Controlling antimicrobial resistance in the hospital. *Infect. Dis. Clin. North Am*., 23(4): 847-864.

Araújo SM, Mourão TC, Oliveira JL, Melo IF, Araújo CA, Araújo NA, Melo MC, Araújo SR and Daher EF (2010). Antimicrobial resistance of uropathogens in women with acute uncomplicated cystitis from primary care settings. *Int. Urol. Nephrol.,* Epub. Available at: http://www.springerlink.com/content/u9217t63117vg2w5/

Costelloe C, Metcalfe C, Lovering A, Mant D and Hay AD (2010). Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ*., 340: c2096.

Drago L, Ripa S, Zampaloni C, De-Vecchi E, Vitali LA, Petrelli D and Prenna M (2005). Activity of ceftibuten, cefaclor, azithromycin, clarithromycin, erythromycin and telithromycin against streptococcus pyogenes clinical isolates with different genotypes and phenotypes. *Chemotherapy*, 51(5): 268-271.

Duerden B, Cookson B, Livermore D, Rowe B, Wall P, Drobniewski F, Watson J and Stanwell-Smith R (1998). Science and Technology-Seventh Report http://www.publications.parliament.uk/pa/ld199798/ldselect/ldsctech/081vii/st0701.htm

Eurosurveillance editorial team (2010). Eurobarometer on antimicrobial resistance highlights areas for action. *Euro. Surveill.*, **15**(15): 19540.

Finkel R, Cubeddu LX and Clark MA (2009). Lippincotts Illustrated Reviews; Pharmacology, 4th Edn., Wolters Kluwer/Lippincott Williams & Wilkins, p.380.

Grivea IN, Al-Lahham A, Katopodis GD, Syrogiannopoulos GA and Reinert RR (2006). Resistance to erythromycin and telithromycin in Streptococcus pyrogenes isolates obtained between 1999 and 2002 from Greek children with tonsillopharyngitis: phenotypic and genotypic analysis. *Antimicrob. Agents Chemother*., **50**(1): 256-261.

Groppo FC, Castro FM, Pacheco AB, Motta RH, Filho TR, Ramacciato JC, Florio FM and Meechan JG (2005). Antimcrobial resistance of Staphylococcus aureus and oral streptococci strains from high risk endocarditis patients. *Gen. Dent*, **53**(6): 410-413.

Gums JG (2002). Assessing the impact of antimicrobial resistance.*Am.J.Health Syst.Pharm*.,**59**(8Suppl.3):S4-6.

Hawser SP, Bouchillon SK, Hoban DJ, Badal RE, Hsueh PR and Paterson DL (2009). Emergence of high levels of extended-spectrum-beta-lactamase-producing gram-negative bacilli in the Asia-Pacific region: data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program, 2007. *Antimicrob. Agents Chemother.*, **53**(8): 3280-3284.

Hsueh PR, Badal RE, Hawser SP, Hoban DJ, Bouchillon SK, Ni Y and Paterson DL; for the 2008 Asia–Pacific SMART Group. (2010). Epidemiology and antimicrobial susceptibility profiles of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia-Pacific region: 2008 results from SMART (Study for Monitoring Antimicrobial Resistance Trends). *Int. J. Antimicrob. Agents,* **Epub.** Available at: http://www.sciencedirect.com/science?

Isturiz R (2008). Global resistance trends and the potential impact on empirical therapy. *Int. J. Antimicrob. Agents,* **32**(Suppl 4): S201-206.

Kim JS, Carver DK and Kathariou S (2006). Natural transformation-mediated transfer of erythromycin resistance in *Campylobacter coli* strains from turkeys and swine. *Appl. Environ. Microbiol.*, **72**(2): 1316-1321.

Lewis R (1995). The rise of antibiotic-resistant infections. FDA *Consumer Magazine*, **29**(7): Available at: http://www.questia.com/googleScholar.qst?docId=5002239353

Malhorta-Kumar S, Lammens C, Coenen S, Van Herck K and Goossens H (2007). Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study. *Lancet*, **369**(9560): 482-490.

Metz-Gercek S, Maieron A, Strauss R, Wieninger P, Apfalter P and Mittermayer H (2009). Ten years of antibiotic consumption in ambulatory care: trends in prescribing practice and antibiotic resistance in Austria. *BMC Infect. Dis.,* **9**: 61.

Mycek MJ, Harvey RA and Champe PC (2000). Lippincotts Illustrated Reviews; Pharmacology, 2nd Edn., Lippincotts Williams and Wilkins, pp.311-320.

Paustian T (1999). The battle against drug resistance. Science Education. Available at: http://www.bact.wisc.edu/Microtextbook/modules.php?op=modload&name=Sections&file=index&req=viewarticle&artid=157&page=1.

Pitout, JD (2010). The latest threat in the war on antimicrobial resistance. *Lancet Infect. Dis.*, **10**(9): 578-579.

Soonthornchaikul N, Garelick H, Jones H, Jacobs J, Ball D and Choudhury M (2006). Resistance to three antimicrobial agents of Campylobacter isolated from organically and intensively reared chickens purchased from retail outlets. *Int. J. Antimicrob. Agents*, **27**(2): 125-130.

Strahilevitz J and Hooper DC (2010). Resistance to other Agents. In: Crossley KB, Kimberly K, Archer JG, Fowler Jr VG Eds. Staphylococci in Human Disease, 2nd Edn., Wiley-Blackwell, UK, p.210.

Straut M, Surdeanu M, Oprisan G, Otelea D and Damian M (1995). Antibiotics and bacterial resistance. A few elements of generic basis for this relationship. *Roum. Arch. Microbiol. Immunol.*, **54**(4): 241-254.

Yague Guirao G, Mora Peris B, Martinez Toldos MC, Rodriguez Gonzalez T, Valero Guillen PL and Segovia Hernandez M (2005). Implication of ermX genes in macrolides- and telithromycin-resistance in Corne bacterium jeikeium and Cornebacterium amycolatum. *Rev. Esp. Quimioter.*, **18**(3): 236-242.