Open Access

Original Article

Susceptibility of Doxycline against S.aureus & S.pneumonia Pak Armed Forces Med J 2020; 70 (Suppl-1): S188-92

RESISTANCE EMERGENCE OF DOXYCYCLINE AGAINST STAPHYLOCOCCUS AUREUS AND STREPTOCOCCUS PNEUMONIAE

Rabia Bushra, Mehwish Rizvi, Yousra Shafiq*, Maqsood Khan, Farya Zafar, Huma Ali***

Dow University of Health Sciences, Karachi Pakistan, *Jinnah Sindh Medical University, Karachi Pakistan, **Ziauddin University, Karachi Pakistan

ABSTRACT

Objective: To determine the in vitro sensitivity pattern of doxycycline against the common pathogens namely *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Study Design: Cross-sectional study.

Place and Duration of Study: Various clinical/pathological laboratories of Karachi Pakistan, from Jun 2016 to Sep 2016.

Methodology: One hundred and ten clinical isolates of *Staphylococcus aureus* and 50 isolates of *Streptococcus pneumoniae* were taken from throat, blood, skin pus, and sputum of patients. Susceptibility of the isolates against the doxycycline was determined through Kirby-Bauer disk diffusion technique. Zone of inhibition appeared around the disks was measured and analyzed as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Data was analyzed using SPSS version 20.

Results: About 65.4% of *Staphylococcus aureus* and 62.0% of *Streptococcus pneumoniae* were found to be sensitive towards doxycycline (p<0.05).

Conclusion: Sensitivity of the doxycycline for *Staphylococcus aureus* and *Streptococcus pneumonia* has been decreasing with time. The drug was observed to possessed little higher activity against *Staphylococcus aureus* than *Streptococcus pneumoniae*.

Keywords: Doxycycline, Disk diffusion method, Microbial resistance, *Staphylococcus aureus, Streptococcus pneumoniae*.

INTRODUCTION

Doxycycline is a broad spectrum antibiotic, belonging to the tetracycline family. The probable mechanism of action is the inhibition of matrix metallo-proteinases (MMPs) expression and inducing proliferation of cells in different cell cultured types. The doxycycline and minocyclin; derivatives of tetracycline, are long acting agents and absorbed well through the gastrointestinal tract. These moieties have shown better anti-*staphylococcal* activity than the parent tetracycline itself at clinically attainable levels[1]. Broad range of the clinical infections including bacteremia, infective endocarditis, osteoarticular, skin and soft tissue, pleura-pulmonary, and device related infections are chiefly caused by *Staphylococcus aureus*[2].

It has been extensively documented that the *Streptococcus pneumoniae* is the causative organism of community-acquired pneumonia (CAP) and also considered to be the main source of sepsis, meningitis, and otitis media. Unfortunately, the resistance emergence of *S. pneumoniae* to the penicillin family consequently results in higher utilization of extended-spectrum cephalosporins, macrolides, fluoroquinolones, and vancomycin. Infectious Diseases Society of America proposed doxycycline to be a substitute remedy for the patients having community-acquired pneumonia[3]. Tetracyclines have been prescribed commonly to manage various skin, throat and blood infections but owing to the progressive resistance, the effectiveness of this group candidate has been diminishing day by day[4].

Correspondence: Dr Rabia Bushra, Department of Pharmaceutics, Dow University of Health Sciences, Karachi Pakistan
Email: rabia_pharmacist@hotmail.com
Received: 05 Feb 2017; revised received: 01 Jul 2017; accepted: 08 Apr 2019

Self-medication and irrational use of antibiotics are thought to be the primary reasons leading to the loss/reduction of antimicrobial response and consequently to the therapy failures. Pathogens have ability to modify their cellular features (natural adaptability mechanism) against antimicrobial agents and so communicate the resistance traits to their offspring[5-8]. However; other contributing common reasons are sub therapeutic doses, unwise and expanded treatment courses especially in developing countries of the world. The present study was conducted to find out the current resistance status of doxycycline against two wide spread clinical isolates namely *Staphylococcus aureus* and *Streptococcus pneumoniae*. Sus-ceptibility of pathogens was estimated by one of the simple, cheap and accurate technique of analysis namely disk diffusion method. The over-all, study was carried out as per official recommendations of CLSI.

METHODOLOGY

A cross sectional study was performed to determine the current in vitro sensitivity pattern of doxycycline against *S. aureus* and *S. pneumoniae* in metropolitan city of Pakistan, Karachi, from June to September, 2016. A total of 160 pathogens including *S. aureus* (n=110) and *S. pneumonia* (n=50) were collected through non-probability consecutive sampling from different clinical laboratories. Patients of aged from 3 to 60 years with *S. aureus* and *S. pneumoniae* infections were included while infants (under 2 years) and elderly aged individuals (>60 years) were ignored. Due to less availability of *S. pneumoniae* during the mentioned study period, the number of tested pathogens was not equal. Pathogens were isolated from the clinical specimens of throat, blood, skin pus, and sputum of patients. Samples were cultured and organisms were identified using standard bacteriological procedures.

Kirby-Bauer antibiotic susceptibility test (Disk diffusion) was chosen to assess the in vitro resistance of the drug doxycycline. Inoculum was prepared by removing few colonies of isolates into the Muller Hinton broth tubes of small volume. These broth tubes were then incubated at 37°C up to six hours to meet the turbidity of 0.5 McFarland standard[9]. Media petri dishes were set by adding sterilized Muller-Hinton agar (Merck) to the same height. Sealed units of doxycycline standard disks (Oxoid, England) were taken from the commercial place. Organisms were streaked well through sterile cotton bud from inoculum suspension. Antimicrobial disks were then placed firmly over the air dried media plates and incubated aerobically for minimum of eighteen and maximum of 24 hours at 37°C. Zones appeared around the disks were measured and analyze by Clinical Laboratory Standards Institute (CLSI) guidelines. According to the official manual pathogens were grouped into sensitive (S), intermediate resistant (IR) and resistant (R)[5].

The in vitro sensitivity pattern of doxycycline against both gram positive bacteria was assessed statistically by applying one sample t-test with 95% confidence interval. SPSS version 20.00 was used where *p*-value ≤0.05 was considered to be statistically significant.

RESULTS

A total of One Hundred Ten *S. aureus* (68.75%) and 50 *S. pneumoniae* (31.25%) organisms were isolated during the study from the pathological laboratories (table-I). These isolates were obtained from different sources including blood, skin pus, stool/urine and sputum (table-II). Chi square test was applied to statistically determine the frequency of infection in various age groups of population. Pathogens were isolated from children of age 3-12 years 42 (26.25%), females 73 (45.62%) and males 45 (28.12%) of age 13 to 60 years. The X^2-value and degree of freedom (df)

Table-I: Breakpoints of Doxycycline (30 µg) against *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Break points	S (mm)	IR (mm)	R (mm)
Staphylococcus aureus	≥16	13-15	≤12
Streptococcus pneumoniae	≥28	25-27	≤24

S: Sensitive, IR: Intermediate Resistant, R: Resistant

were estimated to be 8.198 and 2 correspondingly (p=0.006), showing that the infections caused by these isolates are considered to be non-uniform in all populations (fig-1). The most sensitive age group for *S. aureus* infection was found to between 49 to 60 years while the *S. pneumoniae* was chiefly seen in 3-13 years of age (X^2=19.303, df=4, p=0.000).

Table-II: Sources of clinical isolates.

Source of Pathogens	Samples of *Staphylococcus aureus* (n=110)	Percentage of *Staphylococcus aureus*	Samples of *Streptococcus pneumoniae* (n=50)	Percentage of *Streptococcus neumoniae*
Blood	23	20.91	22	44
Stool/Urine	61	55.45	11	22
Skin pus	26	23.63	9	18
Sputum	-	-	8	16

The in vitro cutoff susceptibility and resistance values as per CLSI guidelines (table-I). Result of one sample t-test showed that the drug appreciably suppressed the pathogens *S. aureus* (t=-38.280, df=31, Mean ± SD = 2.625 ± 1.385, p= 0.000) and *S. pneumoniae* (t=-57.227, df=40, mean ± SD = 2.466 ± 1.457, p=0.000). Fig-2 reflected the activity of doxycycline against both gram positive organisms.

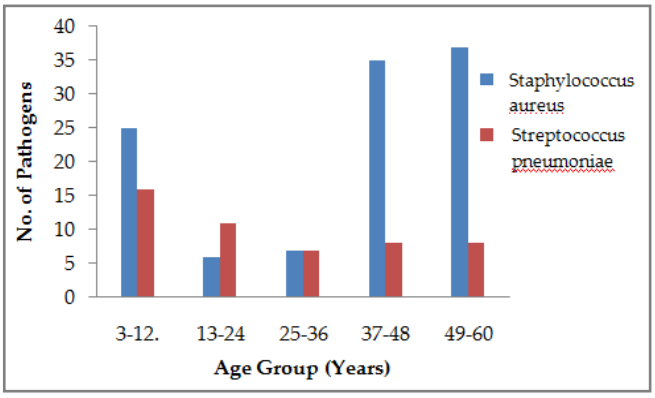

Figure-1: Distribution of Pathogens among various age groups.

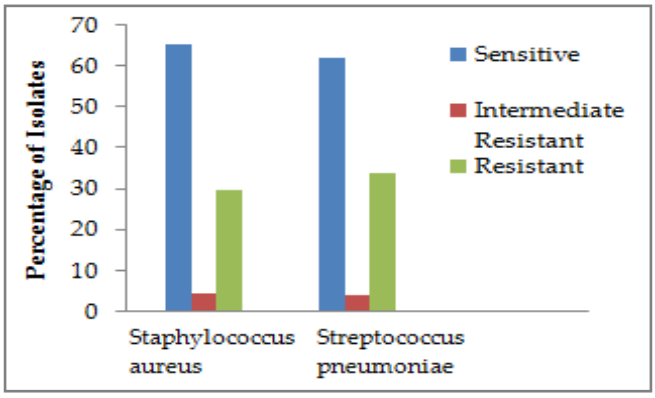

Figure-2: Sensitivity trend of doxycycline against *Staphylococcus aureus* and *Streptococcus pneumoniae*.

DISCUSSION

Treatment failures due to microbial resistance have become a burning concern in various regions of the world[9-11]. However; this problem is being more prevalent in poorly developed and low to middle income countries. Pakistan being a developing country is also a witness of therapy collapse especially when deal with antibiotics. Genetic mutations or the acquisition of mobile genetic elements are considered to be the most common causes of microbial emergence against antibacterial agents. Repeated exposure of such drugs imposes selective pressure consequently acts to be a driving force that ultimately results in the rise and spread of resistant pathogens. Another way of developing resistance is the irrational use not only in patients but in livestock too[6,7]. Various programs has been launched worldwide for clinicians for better patients care and safety leading to ultimate reduction in public hospitalization[8,9] and antibiotics' resistance[10,11].

In the present study a total of 160 gram positive bacterial isolates were recorded, out of which 110 was comprised of *Staphylococcus aureus* and 50 were of *Streptococcus pneumoniae*. The sampling of *S. aureus* and *S. pneumoniae* was made chiefly from the stool/urine (n=61) and blood (n=22) respectively. The samples of both pathogens were collected from male, females and children population of different ages. Current findings revealed

that *Staphylococcus* infections were more prevalent in females (58.0%) rather than males (20.0%) and children (22.0%). While about 66% of *Streptococcus* organisms were mainly isolated from children reflecting the higher occurrence of pneumonia in children. Currently, results of in vitro sensitivity testing revealed that the drug inhibits the growth of clinical strains of *S. aureus* (65.4%) more strongly than *S. pneumoniae* (62.0%). Intermediate resistance (IR) was also determined against the drug in addition to sensitive and resistant zones. About 5 and 33 of 110 *Staphylococcal* isolates exhibited intermediate and complete resistance respectively. IR zones findings are significant from the future prospective showing the possibility to acquire the microbial resistance, moreover; the therapeutic effect is uncertain. *S. pneumoniae* offered less intermediate resistance (4.0%) but more high level of resistance (34.0%) as compared to *S. aureus*. A study was conducted in South West Ethiopia by Mama and co-workers to update the sensitivity pattern of various antibiotics to several bacterial strains of skin wounds. The results confirmed the doxycycline inhibitory response against *S. aureus*[12]. The reported sensitivity of the doxycycline (72.4%) was found to be higher than the present finding, reflecting the regional and racial differences among the individuals belong to different parts of world. Another investigation was made to identify the molecular mechanisms of resistance against the tetracycline group by *S. aureus* in Pakistan. The effectiveness of doxycycline in this study was documented about 60.77%[13] that found to be comparable with the present data. Researchers of many developing countries have also mentioned the ongoing increased in acquisition trend of *S. aureus* against many traditional broad spectrum antibiotics including doxycycline[14,15].

S. pneumoniae is one of the dominant gram positive bacterial strain of upper and lower respiratory tract infections (URTIs & LRTIs) in community[16]. Various combinations of antibiotics have been utilized to treat such infections. Doxycycline, quinolones/fluoroquinolones and macrolides, alone or in combinations are being used for the management of URTIs and LRTIs[17,18]. The present study was made to re-assess the response of doxycycline against *S. pneumoniae*. About 31 clinical isolates have shown the sensitivity, 17 of 50 exhibited high higher resistance and only 2 pathogens exhibited intermediate resistance. An investigation was carried out to determine the resistance of beta lactam and non-beta lactam antibiotics in HIV exposed infants. Authors reported more resistance in non-beta lactam group of chemicals, specifically 19.2% resistance in pneumococcal isolates[19]. Further investigation should be conducted with the identification of MRSA/MSSA *S. aureus* and MDR (multi drug resistant) pneumococcal infections for the confirmation of susceptibility / resistance pattern of the doxycycline.

CONCLUSION

Doxycycline presents a noticeable microbial resistance against the pathogens of *S. aureus* and *S. pneumoniae*. This obvious decline of bactericidal activity is probably owing to the cellular modifications of clinical isolates. However; efforts should be made to control the antibiotic procurement and the judicious utilization of drugs by health professionals as well. Moreover; cooperation from different sectors (medicine, veterinary, horticulture, etc.) is deeming to be necessary to minimize the proliferation and drug resistance especially towards the common pathogens. Future investigations should be planned to explore more grounds for the development of microbial resistance.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Ruhe JJ, Monson T, Bradsher RW, Menon A. Use of long-acting tetracyclines for methicillin-resistant staphylococcus aureus infections: case series and review of the literature. Clin Infect Dis 2005; 40(10): 1429-34.
2. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 2015; 28(3): 603-61.
3. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC. Infectious diseases society of America/American Thoracic society consensus guidelines on the management of

community-acquired pneumonia in adults. Clin Infect Dis 2007; 44 (Suppl-2): S27-S72.
4. Grossman TH. Tetracycline antibiotics and resistance. Cold Spring Harb Perspect Med 2016; 6(4): a025387.
5. Clinical and Laboratory Standards Institute CLSI. Twenty Fourth Informational Supplement 2014; M100-S24.
6. Bushra R, Sial AA, Rizvi M, Shafiq Y, Aslam N, Bano N. Report: Sensitivity pattern of ceftriaxone against different clinical isolates. Pak J Pharm Sci 2016; 29(1): 249-53.
7. Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, et al. The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect 2015; 6(1): 22-9.
8. Malani AN, Richards PG, Kapila S, Otto MH, Czerwinski J, Singal B. Clinical and economic outcomes from a community hospital's antimicrobial stewardship program. Am J Infect Control 2013; 41(2): 145-48.
9. Bishop J, Parry MF, Hall T. Decreasing clostridium difficile infections in surgery: impact of a practice bundle incorporating a resident rounding protocol. Conn Med 2013; 77(2): 69-75.
10. DiazGranados CA. Prospective audit for antimicrobial stewardship in intensive care: impact on resistance and clinical outcomes. Am J Infect Control 2012; 40(6): 526-29.
11. Elligsen M, Walker SA, Pinto R, Simor A, Mubareka S, Rachlis A, et al. Audit and feedback to reduce broad-spectrum antibiotic use among intensive care unit patients: a controlled interrupted time series analysis. Infect Control Hosp Epidemiol 2012; 33(4): 354-61.
12. Mama M, Abdissa A, Sewunet T. Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University Specialized Hospital, South-West Ethiopia. Ann Clin Microbiol Antimicrobiol 2014; 14(1): 13-4.
13. Ullah F, Malik SA, Ahmed J, Ullah F, Shah SM, Ayaz M, et al. Investigation of the Genetic basis of tetracycline. Resistance in Staphylococcus aureus from Pakistan. Trop J Pharm Res 2012; 11(6): 925-31.
14. Tatarkiewicz J, Staniszewska A, Bujalska-Zadrożny M. New agents approved for treatment of acute staphylococcal skin infections. Arch Med Sci 2016; 12(6): 1327-36.
15. Wasihun AG, Wlekidan LN, Gebremariam SA, Welderufael AL, Muthupandian S, Haile TD, et al. Diagnosis and treatment of typhoid fever and associated prevailing drug resistance in northern ethiopia. Int J Infect Dis 2015; 35(1): 96–102.
16. He R, Luo B, Hu C, Li Y, Niu R. Differences in distribution and drug sensitivity of pathogens in lower respiratory tract infections between general wards and RICU. J Thorac Dis 2014; 6(10): 1403-10.
17. Schroeder MR, Stephens DS. Macrolide resistance in streptococcus pneumoniae. Front Cell Infect Microbiol 2016; 6(1): 98-9.
18. Jenkins TC, Sakai J, Knepper BC, Swartwood CJ, Haukoos JS, Long JA, et al. Risk factors for drug-resistant streptococcus pneumoniae and antibiotic prescribing practices in outpatient community-acquired pneumonia. Acad Emerg Med 2012; 19(6): 703-06.
19. Bles P, de Mast Q, van der Gaast-de Jongh CE, Kinabo GD, Kibiki G. Antibiotic resistance of Streptococcus pneumoniae colonising the nasopharynx of HIV-exposed Tanzanian infants. Trop Med Int Health 2015; 20(11): 1559-63.

.....