Original Article

Antimicrobial Resistant Patterns of Pathogenic Bacteria Isolated from Out-Patients Clinics in Mukalla, Hadhramout - Yemen

Eidha A. Bin Hameed[1*]

ABSTRACT

Background: Antibiotic resistance and multi- resistanceof pathogenic bacteria is increasing, becoming a problem for the public health andthreatens lives and increases considerably to healthcare cost.

Objective: To determine the antimicrobial resistant patterns of pathogenic bacteria isolated from the Referred Out-Patients clinics in Mukalla city, Yemen.

Materials and Methods: Specimens were collected from patients at the Referred clinics to private laboratories in Mukalla cityin the period from January 2013 to July 2014. Bacterial isolates wereidentified by conventional diagnostic methods and antibiotic sensitivities testedby the disc diffusion method.

Results: The most prevalent bacterial isolates were *Staphylococcus aureus* (36.3%) followed by *Escherichia coli* (31.1%), *Pseudomonas aeruginosa* (19.0%), *Streptococcus pneumonia*, Proteus species and *Streptococcus pyogens* (3.4%), Klebsiella species and *Haemophillus influenza* (1.7%). Cefuroxime sodium and cefotaxime showed the highest resistance in *Pseudomonas aeruginosa* (90.9%), followed by amoxicillin/clavulanic acid, cefotaxime and cefuroxime sodium in *Escherichia coli* (83.3%, 77.7% and 66.7%) respectively, and cefuroxime sodium and cefotaxime to *Staphylococcus aureus* (71.4% and 61.9%) respectively.

Conclusion: High frequency of bacterial resistance is observed in this study indicating that the antimicrobial resistance is a serious problem.

Key words: Antimicrobial susceptibility, Pathogenic Bacteria, Outpatients.

Bacterial infections continue to become Important causes of morbidity and mortality in developing countries. However, there is a phenomenal increase in antibiotic resistant bacteria which is one of the major problems facing medicine and science today[1]. Rapid spread of resistant microbes affected the effectiveness of antimicrobials and created world-wide problem[2]. The condition is serious in developing countries owing to irrational prescriptions of antimicrobial agents[3]. Despite the importance of antibiotics in human life, very little researches concerning efficacy were carried out in Yemen. This study was essentially designed to determine the antibiotics resistance patterns of pathogenic bacteria isolated from outpatients at the referred clinics in Mukalla city, Hadhramout - Yemen. It is worth mentioning that people in Yemen used to purchase a sub dose according to the money they have.

MATERIALS AND METHODS:
This study was carried out in Mukalla city in a period from January 2013 to July 2014. A total of 384 specimens of med-

1. Faculty of Science, Hadhramout University, Yemen.
*Correspondence to: Dr. Eidha A. Bin Hameed.Faculty of Science.Hadhramout University Tel. +967770847208. E-mail: eidha6@gmail.com

stream urine, ear swab, throat swab, nasal swabs, pus and blood were obtained from outpatients clinicsant tested in private laboratories. Specimens of ear, throat, pus and nasal infection were collected by sterile cotton swabs. Urine and blood specimens were collected in sterile universal containers. The specimens of blood were inoculated into brain heart infusion broth (Oxoid) then sub cultured into the plates of blood agar, MacConkey agar and chocolate agar (Oxoid) which were incubated aerobically at 37˚C for 18-24 hours. Fifty eight strains of bacterial pathogens were isolated and identified from these specimens by conventional diagnostic methods. Antibiotic susceptibility test was carried out using disc diffusion method that has been performed by Clinical Laboratory Standards Institute (CLSI) on Mueller-Hinton agar (Oxoid)[4]. According to the local availability, the antibiotic discs used were cefuroxime sodium (30µg), cefotaxime (30µg), amoxicillin/clavulanic acid (30µg), ciprofloxacin (5µg), amikacin (30µg), lincomycin (15µg) and co-trimoxazole (25µg). A suspension of tested organism was adjusted against 0.5 MacFarland standard turbidity and inoculated into media, then incubated at 35-37 °C for 16-18 hours and examined for evidence the growth.

RESULTS:
The majority of the specimens from which the bacterial pathogenic were isolated were as follows: Ear swabs 11(19.0%), throat swabs 4(6.9%), nasal swabs 3(5.2%), urine 25(43.1%), pus 9(15.5%) and blood 6(10.3%). Aerobic culture of the different specimens yielded different bacterial species as follows: *Escherichia coli* 18(31.1%), *Pseudomonas auroginosa* 11(19.0%), *Streptococus pneumoniae* 2(3.4%), *Staphylococcus aureus* 21(36.3%), Klebsiella species 1(1.7%), Proteus species 2(3.4%), *Haemophilus influnzae* 1(1.7%) and *Streptococcus pyogens* 2 (3.4%) (table 1).
The results of antimicrobial sensitivity test revealed that the most bacterial resistance

Table 1: Frequency of pathogenic bacteria isolated from different specimens

Isolates	Specimens						Total
	Urine	Blood	Ear swab	Nasal swab	Pus	Throat swab	
Staphylococcus aureus	1(4%)	6(100%)	3(27.3%)	2(66.7%)	8(88.9%)	1(25%)	21(36.3%)
Escherichia coli	17(68%)	-	-	-	1(11.1%)	-	18(31.1%)
Pseudomonas aeuroginosa	6(24%)	-	5(45.4%)	-	-	-	11(19.0%)
Streptococcus pneumonia	-	-	-	-	-	2(50%)	2(3.4%)
Streptococcus pyogenes	-	-	1(9.1%)	-	-	1(25%)	2(3.4%)
Proteus species	1(4%)	-	1(9.1%)	-	-	-	2(3.4%)
Klebiella species	-	-	1(9.1%)	-	-	-	1(1.7%)
Haemophilus influenza	-	-	-	1(33.3%)	-	-	1(1.7%)
Total	25(100%)	6(100%)	11(100%)	3(100%)	9(100%)	4(100%)	58(100%)

rates are as follows: Of *Escherichia coli* 83.3% was resistant to amoxicillin/clavulanic acid, 77.8% to cefotaxime, 66.7% to cefuroxime sodium, 55.6% to amikacin and lincomycin, Of *Staphylococcus aureus* 71.4% was resistant to cefuroxime sodium, 61.9% to cefotaxime, and 47.6% to amoxicillin/clavulanic acid. Of *Pseudomonas aeuroginosa* 90.9% was resistant to cefuroxime sodium and cefotaxime, 54.5% to amoxicillin/clavulanic acid and amikacin and 45.5% to co-trimoxazole, while other isolates were non-resistant strains (table 2).

DISCUSSION:

In this study the most prevalent bacterial isolates were *Staphylococcus aureus*36.3%, *Escherichia coli* 31.1%, *Pseudomonas aeruginosa* 19.0%, *Streptococcus pneumonia*, Proteus species and *Streptococcus pyogens* 3.4%, Klebsiella species and *Haemophillus influenzae* 1.7%. Similar findings regarding bacterial pathogenic isolates like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, Klebsiella, and Proteus have been observed by other researchers[5-10]. The present study revealed that *Escherichia coli* was the commonest Gram negative bacteria isolated from urine specimens (68%) this is less than the reported frequency of organisms isolated from urine was *Escherichia coli* comprised 86.02% in Nepal[11], while the prevalence of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* were common accounting for 52%, 11%, 27% and 5% respectively in Tikrit, Iraq[12]. The most prevalent etiological agent was *Escherichia coli* 73.0%, followed by *Proteus* sp. 8.9% and other species of Enterobacteriaceae 9.6% were reported in

Poland[13]. A study carried out in our province revealed that *Escherichia coli* was the most common isolate 35.4%[14]. Examination of pus and blood specimens in our study revealed that the most prevalent organism was *Staphylococcus aureus* comprising six and eight isolates respectively, similar results showed that of 14 organisms obtained from blood specimens, 5(35.7%) isolates were *Staphylococcus aureus*[7], other observations were previously reported of seven isolates were obtained from wound swab, 2(28.57%) of those was *Staphylococcus aureus*[15], other results showed a high number of *Staphylococcus aureus* isolated from pus were 19 and bloodwere14 isolates[5].

Regarding antimicrobial sensitivity test in our study, *Escherichia coli* showed a high frequency of resistance to amoxicillin/clavulanic acid being 83.3%, cefotaxime 77.8% and cefuroxime sodium 66.7%, followed by *Pseudomonas auroginosa* that also showed resistance to cefuroxime sodium and cefotaxime reaching 90.9%. Similarly, *Staphylococcus aureus* also showed markedly resistance to the latter two antibiotics reaching71.45 and 61.95 respectively. This pattern of resistance is comparable to a study carried out locally in which *Escherichia coli* showed high resistance against quinolones with 84.6% and penicillin 78.8%[14] as well as to other studies carried out abroad[16-19]. Also, similar findings regarding drug resistance patterns of *Escherichia coli*, *Pseudomonas auroginosa* have been observed by other researchers[20-23]. Regarding in vitro sensitivity of isolates to different antimicrobial agents, the organism is considered as multi-resistant if it is resistant to three or more antimicrobials[24]. In this study, susceptibility test showed that the multi-resistance rate among the most prevalent

isolates were seen in *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* (Table 2).These results approximately agreed with other reports that showed multi-resistance of *Pseudomonas aeruginosa* and a significant resistance of antibiotics to *Escherichia coli* (24%-54%)[8, 25]. Other studies showed that resistance in *Staphylococcus aureus* was 38.56% and in the Gram negative bacilli was 66.75%[26].Also, another study showed multi-resistance of *Escherichia coli*, *Pseudomonas aeruginosa,* Klebsiella sp. and Staphylococcus sp. to antibiotics[27]. Other studies of Gram negative bacteria reported 85.25% antibiotics resistance, while Gram positive bacteria showed 14.75% antibiotic resistance of the total isolates 65.38% showed multi-resistance [28].

CONCLUSION:

The microbial pathogens, as well as their antibiotic sensitivity patterns may change with time and place. However, the misuse of antibiotics is the leading cause to the emergence of resistance strains of bacteria. Therefore, specific surveillance of resistance patterns of antimicrobial agents is needed to help reforming the healthcare and education systems.

Table 2: Antibiotic resistant patterns of bacterial pathogens isolated

Isolates	Antimicrobial agents						
	Cefuroxime sodium	Cefotaxime	Amoxicillin/ clavulanic acid	Ciprofloxacin	Amikacin	Lincomycin	Co-trimoxazole
Staphylococcus aureus (21)	15(71.4%)	13(61.9%)	10(47.6%)	3(14.3%)	3(14.3%)	7(33.3%)	4(19.0%)
Escherichia coli (18)	12(66.7%)	14(77.8%)	15(83.3%)	6(33.3%)	10(55.6)	10(55.6%)	1(5.6%)
Pseudomonas aeuroginosa (11)	10(90.9%)	10(90.9%)	6(54.5%)	2(18.2%)	6(54.5%)	4(36.4%)	5(45.5%)
Streptococcus pneumonia (2)	2(100%)	1(50%)	-	1(50%)	-	-	1(50.0%)
Streptococcus pyogenes (2)	1(50%)	1(50%)	-	-	1(50%)	1(50%)	-
Proteus species (2)	1(50%)	-	1(50.0%)	-	-	-	1(50.0%)
Klebiella species (1)	-	-	-	-	-	1(100%)	-
Haemophilus influenza (1)	1(100%)	1(100%)	-	-	1(100%)	-	-

REFERENCES:

1. Okesola A.O. Oni A.A. Antimicrobial Resistance among Common Bacterial Pathogens in South Western Nigeria. American-Eurasian J. Agric. & Environ. Sci. 2009; 5(3):327-330.

2. Biadglegne F., Abera B., Alem A. et al. Bacterial Isolates from Wound Infections and Their Antimicrobial Susceptibility Patterns in FelegeHiwot Referral Hospital, North West Ethiopia. Ethiopian Journal of Health Sciences.2009; 19(3):173-177.

3. Roma B., Worku S., Marium S.T. et al. Antimicrobial Susceptibility Pattern of Shigella Isolates in Awassa. Ethiopian Journal of Health Development.(2000); 14(2):149-154.

4. Clinical and Laboratory Standards Institute. Performance standard for antimicrobial susceptibility testing, document M100 – S16. Wayne, PA: 2006.

5. Javeed I., Hafeez R. Anwar M.S.Antibiotic Susceptibility Pattern Of Bacterial Isolates From Patients Admitted To A tertiary Care Hospital In Lahore. Biomedica.2011; 27:19–23.

6. Masoud E.A., Mahdy M.E. Esmat A.M. Bacterial Prevalence and Resistance to Antimicrobial Agents in Southwest, Saudi Arabia. Egypt. Acad. J. biolog. Sci. 2011; 3(1): 105- 111.

7. Samonis G., Rafailidis P.I. Falagas M.E. Antimicrobial susceptibility of Gram negative non urinary bacteria to fosfomycin and other antimicrobials. Future Microbiology.2010; 5(6):961.

8. Al Johani S.M., Akhter J., Balkhy H., et al. 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. 2010; 30(5):364-369.

9. Jones ME., Draghi DC., Thornsberry C., et al.Emerging Resistance Among Bacterial Pathogens In The Intensive Care Unit European And North American Surveillance Study (2000-2002). Ann. Clin. Microbiol.Antimicrob.2004; (3):14.

10. Eltahawy AT. Khalaf RM. Antibiotic Resistance Among Gram Negative Non-Fermentative Bacteria At A teaching Hospital In Saudi Arabia. J. Chemother. 2001. 13:260-264.

11. Thakur P., Ghimire P., Rijal K.R., et al. Antimicrobial Resistance Pattern Of Escherichia coli Isolated From Urine Samples In Patients Visiting Tertiary Health Care Centre In Eastern Nepal. Sunsari Technical College Journal. 2012; 1(1):22-26.

12. Hamada T.A., Mahmood A.R. Ahmed I.A. Antibiotic Resistance In Pathogenic Bacteria Isolated From UTIs In Tikrit Province. Tikrit Medical Journal.2008; 14(1):203-210.

13. Hryniewicza K., SzczypaK., Sulikowska A., et al.Antibiotic Susceptibility Of Bacterial Strains Isolated From Urinary Tract Infections In Poland. Journal of Antimicrobial Chemotherapy.2001; 47:773–780.

14. Al-Haddad A.M., Bin Ghouth A.S.,El-Hosseiny M. Microbial Resistance In Patients With Urinary Tract Infections In Al-Mukalla, Yemen. Sudan J.M.S. 2010; 5,(2):145-150.

15. Weber S.G., Miller R.R., Perencevich E.N. et al. Prevalence of Antimicrobial Resistant Bacteria Isolated From Older Versus Younger Hospitalized Adults: Results of A two-Center Study. The Journal of Antimicrobial Chemotherapy.2009); 64(6):1291.

16. Anguzu J.R., Olila D. Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. Afr. Health Sci. 2007; (3):148–154.

17. Kamberovic S.U. Antibiotic resistance of coliform organisms from community – acquired urinary tract infections in Zenica-Doboj Canton, Bosnia and Herzegovina. J. Antimicrobial Chemotherapy. 2006; 58:344-348.

18. Rahman S., Hameed A., Roghani M.T. et al. Multidrug resistant neonatal sepsis in Peshawar, Pakistan. Arch. Dis. Child Fetal Neonatal.2002; 87:52-54.

19. Khameneh Z.R.,Afshar A.T. Antimicrobial susceptibility patterns of urinary tract pathogens. Saudi J. Kidney Dis. Transpl.2009; 20:251-253.

20. Loureiro M.M., de Moraes B.A., Quadra M.R.R., et al. Study of multidrug resistant microorganisms isolated from blood cultures of hospitalized burns in Rio De Janeiro City, Brazil. Braz. J. Microbiol. 2002;33:73-78.

21. Goosens H., Antibiotic resistance and policy in Belgium. Verh.K. Acad. Geneeskd Belgium.2000;62(5):439-469.

22. El-Astal Z. Bacterial pathogens and their antimicrobial susceptibility in Gaza strip, Palestine. Pakistan J. Med. 2004; 20(4):365-370.

23. Bakare R.A., Oni A.A., Arowojolu A.O. et al. In vitro activity of Pefloxacin and other antibiotics against Gram-negative bacteria in Ibadan, Nigeria. Nigerian Quarterly J. Hospital Med. 1999; 9(1):54-57.

24. Taneja N., Mohan B., Khurana S. et al. Antimicrobial resistance in selected bacterial

enteropathogens innorth India. Indian J. Med. Res.2004;120: 39-43.

25. Maike N., Nathalie R., Tielen S.M. et al. Susceptibility of Pseudomonas aeruginosa Urinary Tract Isolates and Influence of Urinary Tract Conditions on Antibiotic Tolerance. Current Microbiology.2012; 64(1):7-16.

26. Mohanty S., Arti K.B. Dhawan B.D. Bacteriological and antimicrobial susceptibility profile of soft tissue infections from Northern India. Indian Journal of Medical Sciences.Mumbai.2004;58(1):10-15.

27. Radha K., Uma R., Ramanathan G. et al. Evaluation of bacterial isolates from clinical samples for multidrug resistance. J. Microbiol. Biotech. Res. 2012;2(1):138-146.

28. Bhatt C.P., Baidya R., Karki P., et al. Multi Drug Resistance Bacterial Isolates of Surgical Site Infection. Open Journal of Medical Microbiology. 2014; 4:203-209.