Antibiotics resistance among clinical isolates of Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli

Mohammed Hayder Hashim[(1)] and Marwa Hameed AlKhafaji[(2)]

[(1)] *Department of Biology, Al-Farabi University College, Baghdad, Iraq.*
[(2)] *Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*

Abstract

The pattern of antibiotic susceptibility of nine isolate of (*E. coli, P. aerugensa* and *S. aureus)* to five antibiotics ceftazidime (CAZ), azithromycin (AZM), amoxicillin-clavulanate (AMC), ciprofloxacin (CIPR), and cefoxitin (CTX) was determined using disc diffusion method. The results showed that *S. aureus* isolates were 100% resistant all to antibiotics AMC, CAZ, CTX (Extended-spectrum β-lactamases). While 66.6% of isolates were AZM resistant and 33.3% of *S. aureus* isolates resist CIP. All *E. coli* isolate (100%) were resistant to (CTX and CAZ) and 66.6% of *E. coli* isolates were resistant to CIPR and AMC. *P. aerugensa* were 100% resistant to AMC and 66.6% resistant to CAZ, CTX and CIP and 33.3% resistant to AZM.

الخلاصة

نظراً لانتشار المقاومة البكتيرية للمضادات الحياة وماله من تأثير سلبي على حياة الناس والمجتمع.اذ تم اختبار الحساسية للعزلات السريرية لبكتريا *Staphylococcus aureus* و*E.coli, Pseudomonas aeruginosa* لخمس من مضادات الحياة (السيفتازيديم و أزيثروميسين وأموكسيسيلين الكلافولانات وسيبروفلوكساسين و سيفوكسيتين) باستخدام طريقة الانتشار بالأقراص. اظهرت النتائج مقاومة عالية للعزلات البكتيرية حيث كانت مقاومة بكتريا *E.coli* للمضاد الحيوي السيفتازيديم و سيفوكسيتين 100% و 66.6% لكل من سيبروفلوكساسين وأموكسيسيلين الكلافولانات بينما كانت مقاومتها 33.3% للأزيثروميسين. واظهرت النتائج ايضا مقاومةعالية (100%) لبكتريا المكورات العنقودية لكل من السيفتازيديم و سيفوكسيتين و كلافيونيت الأموكسيسيلين وكانت 66.6% من العزلات مقاومة للسيبروفلوكساسين و33.3% مقاومة أزيثروميسين. وكانت بكتريا *P. Aeruginosa* مقاومة ب 66.6% لكلا من السيفتازيديم وسيبروفلوكساسين و سيفوكسيتين بينما كانت مقاومتها للأموكسيسيلين الكلافولانات 100% و33.3% للأزيثروميسين.

1. Introduction

Antibiotic resistance is a main public-health difficulty world-wide, and international works are needed to counteract the problem. There is abundant information on the prevalence of resistance in human pathogens, and these data show that there are considerable geographic differences in the number of resistance to different classes of antibiotics [1]. Antibiotic intake is

progressively was recognised as the main cause of this developing resistance [2]. *Pseudomonas aeruginosa* is an opportunistic pathogen producing severe, acute and chronic nosocomial infections in immune-compromised as well as catheterized or burns of patients, specifically in developing countries; *P. aeruginosa* infections are problematic due to its intrinsic as well as acquired resistance to many effective groups of antibiotics[3]. *S. aureus* infection is a main cause of skin, soft-tissue, respiratory, bone, joint, and endovascular illnesses. The mainstream of these infections occur in persons with multiple risk factors for infection [4, 5]. *Escherichia coli* is a facultative aerobic, rod-shaped, coliform bacterium that is usually present in the lower intestine of warm-blooded organisms (endotherms) [6]. *E. coli* is the major urinary tract pathogen, accounting for 75 to 90% of uncomplex urinary tract infection isolates [7]. The aim of this study was to determine the prevalence of antibiotic resistance to some clinical isolates by studying the antimicrobial susceptibility in Baghdad/Iraq.

2. Methods

2.1 Bacterial isolates

Antibiotic Susceptibility Test was used to determine antibacterial activity against three different bacterial species nine clinical isolates (*S. aureus* , *E. coli* and *P. aeruginosa*), these isolates were obtained from pathology laboratory of biology department, college of Science, University of Baghdad.

2.2 Antibiotic Susceptibility Test

The modified Kirby-Bauer method was used according to [8, 9]. as the following:

2.2.1 Preparation of Mueller-Hinton Plates

Mueller-Hinton agar was prepared according to the manufacturer's instructions, then the medium was cooled to 45-50^{o}C and poured into the plates, allowed to set on a level surface to a depth of approximately 4mm. When the agar was solidified, the plates were stored at 4^{o}C until use.

2.2.2 Inocula Preparation (Turbidity Standard)

For the preparation of the inoculum, colonies from overnight culture of bacterial isolates were transferred to 5 ml tube of normal saline to obtain culture with 1.5×10^{8} CFU/ml by adjusting to 0.5 McFarland standards.

2.2.3 Inoculation of the Test Plate

The plates were inoculated by dipping a sterile swab into the inoculum and the excess of broth was removed from the swab prior to inoculation, by pressing and rotating the swab firmly against the side of the tube above the level of the fluid. The swab was streaked over the surface

of the medium three times rotating the plate through at an acute angle after each application. Finally the swab was passed around the edge of agar surface. The inocula were left for a few minutes to dry at room temperature with the lid is closed. By using a sterile forceps, antibiotic discs were placed on the inoculated medium. Discs should be warmed to room temperature, and then dispensed on the agar surface; and gently pressed down with sterile forceps. The plates were inverted and incubated within 30 min for 18-24 h at 37°C.

2.2.4 Reading the Results

After incubation, the diameters of the complete zone of inhibition were noted and measured in millimetres. The diameter of inhibition zone for individual antimicrobial agent was translated in terms of sensitive, intermediate and resistant categories by comparison with the standard inhibition zone, table (1).

Table (1) Diameter interpretative standards of inhibition zones according to NCCLs

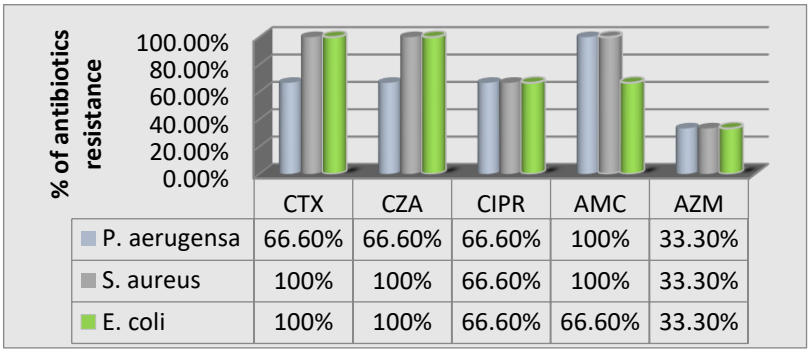
ID	Antimicrobial Agent	Disc Potency	Diameter of inhibition zone (mm)		
			Sensitive	Intermediate	Resistant
	Ceftazidime (CAZ)	**30mg**	**≥18**	**5-17**	**14**
	Azithromycin (AZM)	**15mg**	**≥13**		**12**
	Amoxicillin-clavulanate (AMC)	**30mg**	**≥20**	**-**	**19**
	Ciprofloxacin (CIPR),	**10mg**	**≥21**	**16-20**	**15**
	Cefoxitin (CTX)	**10mg**	**≥18**	**15-17**	**14**

3. Results and discussion

3.1 Antibiotic Susceptibility

The pattern of antibiotic susceptibility of nine isolate of (*E. coli, P. aerugensa* and *S. aureus)* to five antibiotics ceftazidime (CAZ), azithromycin (AZM), amoxicillin-clavulanate (AMC), ciprofloxacin (CIPR), and cefoxitin (CTX) was determined using disc diffusion method according to the guidelines recommended by the National Committee for Clinical Laboratory

Standards (NCCLs). The results of antibiotic susceptibility of these clinical isolates that isolated from different clinical specimens are depicted in figure (1) and figure (2).



	CTX	CZA	CIPR	AMC	AZM
P. aerugensa	66.60%	66.60%	66.60%	100%	33.30%
S. aureus	100%	100%	66.60%	100%	33.30%
E. coli	100%	100%	66.60%	66.60%	33.30%

Figure (1): Antibiogram results of the clinical isolates: *E. coli*, *P. aeruginosa* and *S. aureus to* five antibiotics CAZ, AZM, AMC, CIPR, and CTX)

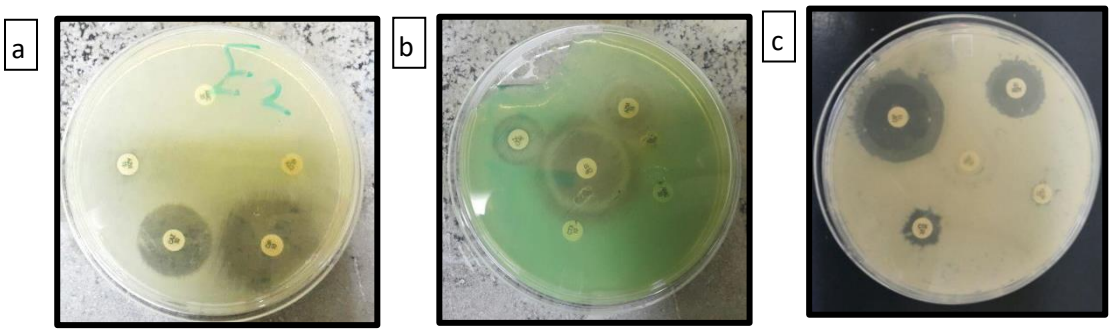


Figure (2): Antibiotic Susceptibility Pattern of clinical a- *E. coli* (E2) b- *P. aeruginosa* (P2) c- *S. aureus* (S2) isolates against various antibiotics according to (Disc- Diffusion Method).

Results of antibiotic susceptibility obtained by this study showed that *S. aureus* isolates (100%) were resistant to AMC, CAZ and CTX antibiotics (Extended-spectrum β-lactamases), while 66.6% , 33% of *S. aureus* isolates were showed resistant against *CIPR* and AZM antibiotic respectively. All *E. coli* isolates (100%) were resistant to CTX and CAZ, this results were strongly similar to the results of local study done by [10], and 66.6% of *E. coli* isolates were resistant to CIPR and AMC. While 100% of *P. aerugensa* resistant to AMC and 66.6% resistant to CAZ, CTX and CIPR, this result somewhat similar to [11], it investigated that ESBLs antibiotics the least effective against *P. aeruginosa* and the resistance reached to 86.0% and 88.3% for CTX and CAZ respectively . This increased in the β-lactam antibiotic resistance isolates among *E. coli*, *P. aerugensa* and *S. aureus* strains can be explained in most cases to the production of β-lactamase enzyme that destroyed the β-lactam ring and inactivated it and this enzyme was encoded by plasmid that easy to transfer among strains [12].

4. Conclusions

Antibiotic resistance has a increased in the last few years. Now the uses of antibiotics are not as effective as they used to be, as none of them had resistance rates less than 33%. Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria.

References

[1] Christgen, B., Yang, Y., Ahammad, S. Z., Li, B., Rodriquez, D. C., Zhang, T., & Graham, D. W. (2015). Metagenomics shows that low-energy anaerobic− aerobic treatment reactors reduce antibiotic resistance gene levels from domestic wastewater. Environmental science & technology, 49(4), 2577-2584.

[2] Cao, B., Qu, J. X., Yin, Y. D., & Eldere, J. V. (2017). Overview of antimicrobial options for M ycoplasma pneumoniae pneumonia: focus on macrolide resistance. The clinical respiratory journal, 11(4), 419-429.

[3] Hajer H. Abdulameer et al.(2018). *Current Research in Microbiology and Biotechnology* 6(3) 1622-1625

[4] Török, E., Moran, E., & Cooke, F. (2016). Oxford handbook of infectious diseases and microbiology. Oxford University Press.

[5] Nazarko, L. (2014). Avoiding admission and facilitating early discharge through OPAT. British Journal of Nursing, 23(Sup14), S30-S36.

[6] Tenaillon, O., Skurnik, D., Picard, B., & Denamur, E. (2010). The population genetics of commensal Escherichia coli. Nature Reviews Microbiology, 8(3), 207.

[7] Foxman, B. (2013). Urinary tract infection. In Women and Health (Second Edition) (pp. 553-564).

[8] Benson, J.M. (2001). Microbiological Applications: Laboratory Manual in General Microbiology. 8th ed. McGraw Hill

[9] Morello, J. A.; Mizer, H. E.; and Granato, P. A. (2006). Laboratory Manual and Workbook in Microbiology Applications to Patient Care. 18th ed. McGraw Hill

[10] Yaseen N & Al-Mathkhury H (2015) Effectiveness of Some β-lactamase Encoding Genes on Biofilm Formation and Slime Layer Production by Uropathogenic Escherichia coli. World J Exp Biosci 3: 61-68.

[11] Asmaa AALK, Amina NALT & Rabab QALS (2015) Incidence and Antibiotics Sensitivity of Multidrug-Resistance of Pseudomonas aeruginosa Isolated from Burn's

Patients and Environmental Samples fromThree Hospitals in Baghdad. Jornal of Biotechnology Research Center 9: 67-73

[12] Brooks, G.F.; K.C. Carroll; J.S. Butel; and S.A. Morse.(2007).Jawetz, Melnick and Adelbergs Medical Microbiology. 24th.ed. The McGraw-Hill Companies, Inc., New York.P.224-232.