Isolation and Identification of Some Types of Bacteria Isolated from Different Pathological Conditions and Study of their Resistance to Antibiotics

Samara Salam Al-Fahdawi[1], Hana Abdel Latif Yassin[2] , Nagam Khudhair[3]
[1,2,3] College of education for girls/University of Anbar/Iraq
Email: sam20w4006@uoanbar.edu.iq
Email: edw.hanatreek@uoanbar.edu.iq
Email: nagam.mahdi@yahoo.com

Abstract
Infectious illnesses acquired in hospitals, known as nosocomial infections, caused by bacteria that have developed resistance to several antibiotics are a major public health concern around the worldThis bacteria is a common contaminant in healthcare facilities, where it has emerged as a powerful human pathogen. The goal of this research is to examine the antibiotic resistance patterns of two different species of bacteria—K.pneumoniae and A.baumannii. Since 170 human clinical samples were gathered from a variety of locations, we may say that (blood, pneumonia, wounds, urine). Bacterial culture, microscopic, and biochemical examinations, as well as examination with the Vitec device, revealed that 35 isolates exhibited characteristics typical of K.pneumoniae and that 16 isolates belonged to the type A.baumannii; subsequent testing of these isolates for resistance and sensitivity to 10 antibiotics, via the diffusion around the discs method, revealed that 33 K.pneumoniae and 9 A.baumannii isolates were resistant to Cefazolin, while baumannii is resistant to it, and 11 K.pneumoniae and 8 A.baumannii isolates showed resistance to the anti-piperacillin, while 5 isolates of each type showed resistance to the anti-Imipenem, and 10 K.pneumoniae and 6 A.baumannii isolates were resistant to Gentamicin, and 9 K.pneumoniae and 8 A.baumannii isolates were resistant to Ciprofloxacin, and 16 There were 24 K. pneumoniae and 9 A. baumannii isolates that were resistant to trimethoprim, and 5 K. pneumoniae and 11 A. baumannii isolates that were resistant to levofloxacin antagonist Tigecycline.
Keywords: isolation, diagnosis, K.pneumoniae, A.baumannii, antibiotic resistance.

1. Introduction

Klebsiella bacteria, which are part of the family Enterobacteriaceae and were first identified by German scientist EdwanKlebs in 1834 and named after him, were first diagnosed by scientist Fried Landers in 1882 and were subsequently dubbed Fried Landers Bacilli. Many different types of diseases in humans, animals, and plants are traced back to this particular contaminant, making it one of the most significant polluted microorganisms. It is also an opportunistic gram-negative bacterium that has been linked to numerous hospital-acquired infections such as UTIs, pneumonia, and others (1). Gram-negative bacilli of the genus A.baumannii can be found virtually anywhere and are easily isolated from the skin, mucous membranes, and a hospital setting (2). They pass the catalase test but fail the oxidase test; they also lack motility. It occurs naturally as an opportunist and is responsible for a wide variety of hospital-acquired illnesses. Infections in the respiratory tract, the skin, and the urinary tract are other common results (3-4). A. baumannii bacteria are dangerous because they produce a number of virulence factors—including cytotoxins, aerobactin, and curly fiber—as well as the toxins colicin V and capsular, and the hydrolytic enzymes lipase and protease. Protease)) (5). The purpose of this research is to examine the prevalence of antibiotic resistance among several strains of bacteria, including Acinetobacterbaumannii and Klebsiellapneumoniae.

2. Materials and Methods

Collect of specimen

From October 20, 2021, to January 10, 2022, pathologists in Anbar Governorate collected 170 clinical specimens from a wide variety of pathological cases. The samples were from both the Ramadi Hospital for Women and Children and the Ramadi General Hospital, and they covered a wide range of conditions, from respiratory infections to blood clots to wounds. After 24 hours at 37 °C in differential and selective media (blood agars and Maconkey agar , respectively), the samples were purified on the nutrient agar medium to obtain pure colonies.

Isolation and identification of bacteria

The producing isolates were subjected to a variety of diagnostic and biochemical assays, as recommended by various bacterial diagnosis reference materials (6). After a diagnosis was made with the Vitec device, all of the resulting bacterial isolates were tested for resistance and sensitivity to

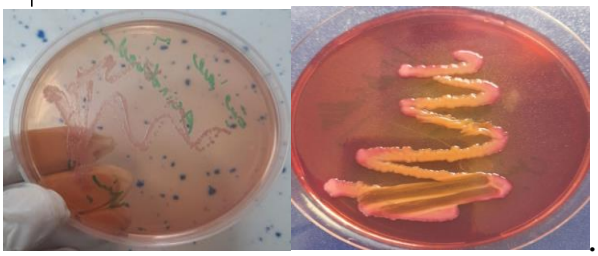
doi.org/10.31838/hiv23.02.91 Received: 20.10.22, Revised: 01.11.22, Accepted: 07.12.22.

10 drugs at varying doses (Cefazolin, Ceftazidime, Ceftriaxone, PiPeracillin, Imipenem, Genetofgetamicin, Tiprofloxacin, Ciprofloxacin, Levetrimicin, and Levetrimicin). The widths of the inhibition zones surrounding each disc were measured and compared with the accepted international measurements based on the method of diffusion around the discs (Disc Diffusion Method) using HentonAkar's mullein medium.

3. Results and Discussion

Isolation and diagnosis

The bacteria were cultured on MacConkey medium, resulting in convex, smooth, shiny, pink colonies with a mucous consistency, and the biochemical and microscopic examinations showed that they are short and Gram-negative bacilli, positive for each of the catalase test, consumption of citrate and fuchsia proscur, and negative when this was done by the MADIZ test and motility test. Examination with the Vitec device, where the final results of the diagnosis showed that 35 bacterial isolates bore the characteristics of K.pneumoniae bacteria, as in Figure (A-1), and another bacterial type was isolated, A.baumannii, and its colonies on blood vessels were characterized as convex and light leaden or white. Not analyzed for blood. As for the Maconki acres, the bacterial colonies appear small, raised, creamy, smooth, and some of them showed a viscous, pale and non-Lactose fermentation (7) as in Figure (1-B). The reason for the absence of more bacterial isolates may be due to the possibility of antibiotic treatment, which may have a significant effect in reducing the proportions of isolated bacteria.



K.pneumoniae, B- A.baumannii growing on Maconky medium.Figure 1:A

Consistent with our research results, study (8) discovered that the greatest proportion of K.pneumoniae isolates came from urinary tract infections and wounds, while study (9) found that the highest proportion of A.baumannii isolates came from urinary tract infections, which is in line with our current study's finding that the highest proportion of isolates (11 isolates) of this specie came from urinary tract infections. These bacteria are considered opportunistic pathogens because they are linked to infections that patients and inpatients acquire from the outside world; the hospital environment facilitates the spread of these bacterial types because of the abilities of one species in particular to thrive in such an environment.

The sensitivity of bacteria to antibiotics

In order to determine how sensitive Gram-negative bacterial isolates were to ten different antibiotics, they were plated out on Mueller-Hinton agar plates in accordance with the (Kirby-bauer method), as modified and reported by the World Health Organization (10Table (1) shows that the highest resistance of K. pneumoniae was (94.28%) towards the antibiotics Ceftriaxone and Cefazolin, and this result is consistent with the result of the researcher, as it reached (11.1) 100%. This suggests that there was a wide range of antibiotic sensitivity and resistance among the isolates studied. And, consistent with the study, they are also (80%) more sensitive to the antagonists Imipenem and Tigecycline (12). Our results showed that this bacteria was resistant to Ceftazidime at a rate of 77.14%, which was consistent with the findings of the original study (13), while the anti-Trimethoprim strain was resistant to it at a rate of 68.57% and to Levofloxacin at a rate of 45.71%, which was not supported by the original study. Resistant rates to Piperacillin, Gentamicin, and Ceprofloxacin were all about the same at 31.42 percent, 28.57 percent, and 25.71 percent, respectively.

Table (1) Percentage of antibiotics

P VALUE	Chi-Square	R	I	S	Antibiotic
0.000	27.457	33 94.28%	0%	2 (5.71%)	Cefazolin
0.001	10.314	27 77.14%	0%	8 22.85%	Ceftazidime
0.000	27.457	33 94.28%	0%	2 (5.71%)	Ceftriaxone
0.015	27.457	11 31.42%	5 14.28%	19 54.28%	PiPeracillin
0.000	34.686	5 14.28%	2 5.71%	28 80%	Imipenem
0.011	6.429	10 28.57%	0%	25 71.43%	Gentamicin
0.029	7.086	9 25.71%	7 20%	19 54.28%	Ciprofloxacin
0.000	24.029	16 45.71%	3 8.57%	16 45.71%	Levofloxacin
0.000	34.686	5 14.28%	2 5.71%	28 80%	Tigecycline
0.028	4.829	24 68.57%	0%	11 31.43%	Trimethoprim

This bacterium's resistance to antibiotics suggests the presence of efflux mechanisms, which cause the antibiotic to leak out of the cells (15). Results were 68.2 %, which is in line with an Al-Ani study (17) The frequent use of these antagonists or the validity of the health of what is not correct and not correct may be among the causes of high resistance, as (18) ASKOURA (2011) indicated the development and spread of the multiple anti -vital anti -anti -anti -anti -anti -anti -livestocks, which is Muhammad on the plasma moving between the pathological bacteria. Conjugation mediated by the plasmid is the mechanism through which bacteria acquire and transmit resistance to drugsThe study found that P. pneumoniae.K was more resistant to Ceftriaxone, which agrees with the findings of the previous study (11). The presence of the AmpC enzyme B-lactamases, which destroys all beta-lactam rings of B-

lactams except cefepime and carbapenem, suggests the development of bacterial resistance to antibiotics and the evolution of resistance mechanismsThe results demonstrated Gentamicin resistance due to the presence of a number of narrow-activity enzymes that can render this antigen ineffective, and the outcome of the investigation for this antibody matched the outcome of the researcher's experiment (19)High resistance was found in the investigation of the antagonist Ceftazidime, which was confirmed by the results of the present study (13). And that ran counter to the researcher's finding (20), which totaled 38.4%. Broad-spectrum beta-lactam enzyme synthesis is associated with resistance to this antibody because these enzymes inactivate the antibody (21)Despite the researcher's conclusion (16) that 100% of the sample population was immune to PiPeracillin, the actual percentage of resistant isolates was 31.42%This finding is in line with the conclusion that an imipenem antagonist has a high level of efficacy against bacterial isolates (22)The resistance rate to ciprofloxacin was 25.71%, and this result is contrary to what was reached by (23), as the resistance rate reached 72.3%. The reason for the sensitivity of this bacteria to this type of antibiotic may be due to the interference of the antibody with the enzymes responsible for the process of reproducing DNA, and thus stopping the protein manufacturing process. (24) Levofloxacin showed a resistance rate of 45.71%, which is inconsistent with what the researcher reached (14), as it reached 82.5% As the results of Table (2) showed, the highest resistance of A.baumannii isolates was to both antibiotics Ceftriaxone and Tigecycline by (68.75%), and more sensitive to Imipenem and Gentamicin. 25). The resistance to Levofloxacin was at a rate of (62.5%), while the antibiotics Cefazolin, Ceftazidime, and Trimethoprim gave the same results for the resistance at a rate of (56.25%), while the results of the antibiotics Piperacillin and Ciprofloxacin were also the same, which is (50%).

Table (2) Percentage of antibiotics

P VALUE	Chi-Square	R	I	S	Antibiotic
0.144	3.875	9 56.25%	3 18.75%	4 25%	Cefazolin
0.617	0.250	9 56.25%	0%	7 43.75%	Ceftazidime
0.134	2.250	11 68.75%	5 31.25%	0%	Ceftriaxone
NS	NS	8 50%	0%	8 50%	PiPeracillin
0.646	0.875	5 31.25%	7 43.75%	4 25%	Imipenem
0.046	4.00	6 37.5%	4 25%	6 37.5%	Gentamicin
0.305	2.375	8 50%	3 18.75%	5 31.25%	Ciprofloxacin
0.317	1.00	10 62.5%	0%	6 37.5%	Levofloxacin
0.134	2.250	11 68.75%	0%	5 31.25%	Tigecycline
0.617	0.250	9 56.25%	0%	7 43.75%	Trimethoprim

The rate of bacterial resistance to antibiotics because this species has the ability to collect multiple genes to resist a group of antibiotics, which leads to the development of new strains that are resistant to drugs on a large scale, and among these resistances that are in the form of modifications in the channels of the cell wall, and the flow pumps, as work is done. This affects the extrusion of the antibiotic from the inside of the cell to the outside (26). Also, the occurrence of mutations in the proteins of the channels of the cell wall can impede the passage of antibiotics to the medium surrounding the plasma, or may decrease the expression of the efflux pumps that lead to bacterial metabolism, which leads to a decrease in expressionStudies have shown that A.baumannii has the ability to resist many antibiotics, especially beta-lactam antibiotics (27). As the researcher showed (28) in her study, which she conducted on 22 local isolates of A.baumannii, where the percentage of resistance to Ceftazidime antibody was 100%, and it does not agree with the result of our current study, where the percentage of resistance to this antibody was 56.25%The rate of resistance to Trimethoprim in this study was close to the study conducted by the researchers (29), as well as close to the result of the researchers (30)As for the result of the antagonist Ceftriaxone, it was contrary to what the researcher reached (31), as it was 100%The results of the resistance of this type of bacteria to anti-Gentamicin were close to the study conducted by the researchers (32)The results of the current study of ciprofloxacin resistance agreed with the findings of the researcher (33), which found that the percentage of resistance to this antidote reached 60%While all the isolates were more sensitive to Imipenem, and this may be due to the lack of use of this antidote, and this result was similar to what was reached (17), and similar to the researchers' study (34), and to the study (31), and is not compatible with the study (26)The result of the resistance to the anti-Piperacillin of the group of anti-B-lactams was close to what the researcher (35) concluded in his study

It was shown (36) and others who obtained resistance genes through horizontal transmission and mutations of A.baumannii bacteria

References

1.Chan, Y.; Liu, J.; Pociask, D.; Zheng, M Mietzner, T. and Berger, T (2002)
Lipocalin 2 is required for pulmonary host defense against Klebsiella infection. J Immunol. 182(8) 47 – 56
Brooks , G. F.; Carroll,K.C.; Butel , J. S.; Morse, S. A.; Mietzner,T.H - 2 .(2016)Jawetz, Melnick, &Adelberg'sMedical Microbiology, 26th ed. A lange medical book
Sundar, S.K.; KumariPushpa Rani, T.P - ;.3 Vijayalakshmi, B. and Murugan, M. Isolation and 16S rRNA Sequencing of Clinical Isolates of Acinetobacterbaumanii. International Journal of Current Microbiology and Applied Sciences. 3 .2014 .858-855 :(5)

Momtaz, H.; Seifati, S. and Tavakol, M4 - . Determining the Prevalence and Detection of the Most Prevalent Virulence Genes in Acinetobacterbaumanniiisolated from Hospital Infections. International Journal of Medical Laboratory. .2015 .97-87 :(2)2
Abdulla, A.A.; AL Thahab, A.A.; Abed5 - , T.A.; Mahdi, R. K. and Fadhil, S. Screening of virulence factors in Acintobacter baumannii isolated from clinical samples. International Journal of Current Research and Academic Review. 3 (6): 128-134. .2015
MacFaddin, J.F.)2000(Biochemical Tests for6 - Identification of Medical Bacteria. 3rd edition Lippincott, Williams and Wilkins. Philadelphia. London.
Jawetz, E.; Melnick, J. A. and Adelberg, E. A. (2001). Review of - 7 Medical microbiology 22nd ed .Appleton and Lange: 704 pp
8. Al-Zubaidi, Muhammad Mahdi Abdul-Mohsen (2012). The role of plasmids in the production of bacteriocin from Kleb.spp isolated from clinical samples. Master Thesis, Institute of Genetic Engineering and Biotechnology, University of Baghdad
Jabur, M.H. Isolation of Acinetobacter -9 baumannii from different clinical source and study some antibiotic resistant and β-lactamase production. Medical Journal of Babylon. 11(2):456-464.2014
10- Vandepitte, J.; Verhaegen, J.; Engbaek, K.; Rohner, P.; Piot, P.; Heuck, C. (2003)." Basic laboratory Prodceduresin Clinical Bacteriology ". 2nd ed .WHO. Geneva
11. Al-Fahdawi, Khaled Ibrahim Mahmoud Abd (2020). Susceptibility of some pathogenic bacterial species towards locally isolated and molecularly patterned bacteriophages
Nkene, I. H., Ngwai, Y. B., Bassey, E. B., Abimiku, H., Ibrahim, T - & ,.12
Yahaya, I. (2020). Molecular Characterization of Escherichia coli with Ciprof-loxacin and Extended-Spectrum Cephalosporins Co-resistance from Patients Attending Tertiary Hospitals in Nasarawa State, Nigeria. GSJ, 8(5)
13. Al-Fahdawi, Ahmed Ghazi Hamad Ahmed (2021). Effect of gold nanoparticles on gene expression of some cell wall and capsular genes in antibiotic-resistant Klebsiella pneumonia
14. Haqqi, Ismail Jassim (2021). Isolation and identification of Klebsiellapneumoniae bacteria that cause pneumonia and identification of some of its virulence factors by PCR technique
-Mofolorunsho, K. C., Ocheni, H. O., Aminu, R. F., Omatola, C. A& ,.15.
Olowonibi, O. O. (2021). Prevalence and antimicrobial susceptibility of extended-spectrum beta lactamases-producing Escherichia coli andKlebsiellapneumoniae isolated in selected hospitals of Anyigba, Nigeria. African Health Sciences, 21(2), 505-512
16. Al-Zinkannah, Iman Abbas Ali Nourallah 2012). Bacteriological and genetic study of Klebsiellaspp isolated from various pathological infections
17. Al-Ani, NidaaYassinTaha (2021) A study of the bacterial diversity that causes inflammation of caesarean section wounds in the city of Ramadi. A master's thesis submitted to the College of Science | University of Anbar
18. Askoura M, Mottawea W, Abujamel T, Taher I. Efflux pump inhibitors (EPIs) as new antimicrobial agents against Pseudomonas aeruginosa. Libyan J Med. 2011;6(1)
19. Al-Abdali, Intisar Hamid Ali 2019). Effect of extracts of fig fruits, Ficuscarica L., and olive, Oleaeuropaea L., on some types of clinically isolated pathogenic bacteria. A master's thesis submitted to the Council of the College of Education for Girls - Anbar University
20. Conscientious, HishamNazim 2019). A comparative study of the susceptibility of Klebsiellaspp bacteria isolated from clinical and environmental samples
Bonnet,R.;Marchndin,H.;Chanal,D.Sirot,D.Labia,R.;DeChamps,C.;21.
umas-Bilak,E.andSirot,J.(2015). Chromosome-Encoded class D- ß-lactamase OXA-23 in Proteus mirabilis . Antimicrob. Agen. Chemother. 46(6):2004 -2006
Nkene, I.H., Ngwai, Y.B., Bassey, E.B., Abimiku, H., Ibrahim, T& ,.22.
Yahaya, I. (2020). Molecular Characterization of Escherichia coli with Ciprof-loxacin and Extended-Spectrum Cephalosporins Co-resistance from Patients Attending Tertiary Hospitals in Nasarawa State, Nigeria. GSJ, 8(5)
23. Al-Dulaimi, Hassan Hilal Rashid 2016). Investigation of some virulence factors for some pathogens of urinary tract infection using polymerase chain reaction. Master Thesis, College of Science, University of Anbar
-Masadeh, M. M., Alzoubi, K. H., Al-azzam, S. I., Khabour, O. F., & Al24.
buhairan, A. M. (2016). Ciprofloxacin-induced antibacterial activity is atteneuated by pretreatment with antioxidant agents. Pathogens, 5(1), 28
25. Al-Hadidi, Osama Imad, NajdatBahjat, Ibrahim Salih Al-Jubouri (2019) Isolation and diagnosis of newly isolated Acinetobacterbaumannii from patients in Kirkuk hospitals and studying its resistance to antibiotics, Kirkuk University Journal/Scientific Study (KUJSS) Volume (14), (Issue 3), pp(155-173

26. NK. Tektook, A. A. Mohammed, E. Y. Pirko. "Anti-bacterial Susceptibility Patterns ofAcinetobacterbaumannii Isolated From Urine of Pregnant Women in BaghdadDiyala", Journal For Pure Science, 13(1-part 2),12 (2017

-Moradi, J.; Hashemi, F. B. and Bahador, A. (2015). Antibiotic27.
Resistance of Acinetobacterbaumannii in Iran: A Systemic Review
of the Published Literature. Osong Public Health and Research
Perspectives, 6(2): 79–86

28. Al-Mashhadani, Enas Ibrahim Jassim 2010). Studying the effectiveness of bacteriocin produced by Lactobacillus plantarum on the virulence factors of Acinetobacterbaumannii. Master's thesis, College of Science, Al-Mustansiriya University: 120 pages

29. A. H. Al-Hamadani, A. M. Al-Mohana, A. S. Al-Khazaali, "Emergence of plasmid
mediated aac (6)-Ib-cr Gene in Flouroquinolon-resistant Acinetobacterspp", Al-
Qadisiah Medical Journal, 10(17), 96 (2014)

-Daryanavard, R. and Safaei, H. S.(2015). Virulence genes and30.
antimicrobial resistance properties of Acinetobacterbaumannii
isolated from pediatrics suffered from UTIs. International Journal of
Advanced Research in Biological Sciences, 2(11): 272–279

-31. Taha, Raghdalyad (2018). A genetic and molecular study of Acinetobacterbaumannii isolated from different clinical sources and its relationship to phages in Diyala Governorate. Master Thesis, College of Science | University of Diyala

-Al-Masoudi, K. K.; Al-Saffar, J. M. and Kendla, N. J. (2015.(32.
Molecular characteristics of Multidrug Resistant Acinetobacterbaumannii Isolated from Baghdad. Hospitals Iraq journal of Science,
1562) B): 1394-1399.

33. Ahmed, RashaZiyad Tariq (2011). A study of the phenotype and genetic pattern of some virulence factors of Acinetobacterbaumannii isolated from different clinical cases

34. MF Lin, CY Lan. "Antimicrobial resistance in Acinetobacterbaumannii: From bench
to bedside", World Journal of Clinical Cases, 16 (12),787 (2014.(

-35. Mohamed, Mohamed Yassin (2016). Design and use of antimicrobial peptides to combat antibiotic-resistant Acinetobacterbaumannii. Master Thesis, Institute of Genetic Engineering and Biotechnology for Graduate Studies

-36 Hu S, Niu L, Zhao F, Yan L, Nong J, Wang C, et al. Identification
of Acinetobacterbaumannii and its carbapenem-resistant gene bla
OXA-23-like by multiple cross displacement amplification
combined with lateral flow biosensor. Sci Rep. 2019;9(1):1–11.