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Prevalence of *Proteus spp.* in some hospitals in Baghdad City

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Abstract:
In this research, 152 clinical samples were collected from different hospitals in Baghdad city, 30 isolates of *Proteus spp.* were identified from urine, wounds and burns by using different bacteriological and biochemical assays. It was found that 20 (66.6%) samples were identifies as *Proteus mirabilis* and 10 (33.3%) samples were *Proteus vulgaris*. Among the 30 isolates of *Proteus spp.*, 18 isolates (60%) were isolated from urine samples; 7 (23.3%) isolates from wounds samples and 5 (16.6%) isolates from burns samples. Out of 20 isolates of *P. mirabilis*, 13 (65%) isolates were from urine samples, 4 (20%) isolates were isolated from wounds samples and 3 (15%) isolates from burns. According to the gender, out of 30 *Proteus spp.*, 17 (56%) were from female and 13 (43%) from male at different age.

Keywords: Cefotaxime resistance *Proteus*, CTX-M-2, ESBLs producers.

انتشار بكتيريا *Proteus spp.* في بعض مستشفيات مدينة بغداد

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الخلاصة:
في دراسة شملت عدد من مستشفيات مدينة بغداد، تم جمع ١٥٢ عينة سريرية مختلفة تضمنت عينات من الادرار، والحروق، والجروح. تم تشخيص ٣٠ عزلة من جنس بكتيريا *Proteus* باستخدام الاوساط البكتريولوجية والاختبارات البايوكيميائية، شُخصت ٢٠ عزلة (66.6%) لنوع *Proteus mirabilis* و ١٠ عزلات (33.3%) لنوع *Proteus vulgaris*. كذلك تم عزل جنس الـ *Proteus* بواقع ١٨ عزلة (60%) من عينات الادرار، و ٧ عزلات (23.3%) من الجروح ، و٥ عزلات (16.6%) من الحروق. كانت نسبة عزل بكتيريا *P. mirabilis* من العينات السريرية قيد الدراسة بواقع ١٣ عزلة (٦٥ %) تم عزلها من عينات الادرار، و٤ عزلات (20%) من عينات الجروح، و٣ عزلات (15%) من عينات الحروق. وفقاً لجنس المصاب تم عزل ١٧ عزلة (56%) من *P. mirabilis* من اصابات الذكور بينما كانت نسبة عزل البكتيريا من اصابات الاناث (43%) بواقع ١٣ عزلة.

Introduction

Proteus is Gram negative, facultative anaerobic, rod shaped bacteria. It has swarming motility, urease activity, do not usually ferment lactose**.** Since it belongs to the family of *Enterobacteriaceae*, general behaviors are applied on this genus: It is actively motile, non-spore forming, non-capsulated oxidase-negative, but catalase and nitrase positive. To identify *Proteus*, specific tests including positive urease and phenylalanine deaminase tests [1-3] were used. *Proteus* is widely distributed in the natural environment. It can be found in polluted water and in soil and manure, where it plays an important role in decomposing organic matter of animal origin [1, 4].

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The genus *Proteus* currently consists of five named species (*P. mirabilis, P. penneri, P. vulgaris, P. myxofaciens, and P. hauseri*) and three unnamed genomo-species (Proteus genomo-species 4, 5, and 6), *P. vulgaris, P. mirabilis* and *P. penneri* are opportunistic human pathogens. [5, 6].

P. mirabilis is often found as free-living organisms that is often found in soil, water, and the intestinal tract of many mammals, including humans [3, 7]. On the species level, indole is considered reliable, because it is positive for *P. vulgaris* but negative for *P.mirabilis* [1, 2]. *P. mirabilis* is the third most common (after *E.coli* and *Klebsiella pneumoniae*) cause of complicated UTI (causing 12% of infections) and the second most common (after *Providencia stuartii*) cause of catheter-associated bacteriuria in the group of long-term catheterized patients (causing 15% of infections) [7].

It is more commonly associated with urinary tract infections (UTIs) which can be subdivided into two categories: hematogenous infections (also known as systemic infections) and ascending infections, in which bacteria colonize, step by step, the introitus, urethra, bladder, ureter, and, in the end, the kidneys. The second type of UTI is more common to *Proteus* strains, and infections with urinary catheters in place or with structural abnormalities [4]. Besides UTI, *P. mirabilis* have been described as opportunistic etiological agents in infections of the respiratory tract and wounds, burns, skin, eyes, ears, nose, and throat, as well as in gastroenteritis resulting from the consumption of contaminated meat or other food [8, 9].

P. mirabilis possesses a host of potential virulence factors that may aid its pathogenesis, including hydrolysis of urea by urease, cell invasiveness and colonization due to swarming, cytotoxicity by hemolysins, cleavage of IgA and IgG by protease, production of biofilm and adherence to the uroepithelium mediated by five types of fimbriae that have been described to date for *P. mirabilis,* [9, 10].

Materials and methods

Isolation and identification

Through the period from September 2013 to March 2014, 152 clinical samples of urine, wounds, and burns were collected from patients attending to Al- Yarmook teaching hospital, Al-Nu`man hospital, Teaching labroteties/ Baghdad medical city and Saint Raphael hospital. Samples were transferred to the lab for isolation and identification of *Proteus* .by using sterile equipment and media.
All samples were streaked on Blood agar, MacConkey agar and XLD agar plates. The plates were incubated aerobically at 37^{o} C for 24 hours.

The isolates were identified depending on the microscopical feature by using Gram stain to detect their response to stain, shape and arrangement [11, 12]. In addition, the morphological features on culture media such as Swarming on blood agar, Non lactose fermented growth on MacConkey agar and colourless growth on Xylose lysine deoxychoclate agar (XLD) agar were examined, also several of biochemical assays were used to identify the *Proteus* isolates, such as catalase, oxidase tests, Indole, Methyl Red/ Voges- Proskauer (MR-VP) test, citrate utilization tests, Urea test, Motility test, gelatin liquefaction test and triple sugar iron agar test [2].

Antibiotic susceptibility test

Kirby-Bauer method was done according to [2] to carry out antibiotic susceptibility test for 25 different antibiotics. A sterile cotton swab was submerged into bacterial suspension standardized to match the turbidity of the 0.5 McFarland turbidity standard (1.5 x 10^8 CFU/ml) by preparing serial dilutions of 18 hrs. Brain Heart Infusion culture of tested bacteria, third dilution was used after comparing it with the 0.5 McFarland turbidity standard.
The surface of Mueller Hinton agar plates were spreaded into four directions by the bacterial suspension, the plates were left for 10 min. to dry. Then, the antibiotic disks were placed by sterile forces on the agar and pressed firmly to ensure the contact with the agar. The plates were inverted and incubated at 37^{o}C for 18-24 hrs.

Inhibition zones developed around the antibiotic disks were measured by using a metric ruler in millimeters according to Clinical Laboratories Standards Institute (CLSI, 2011). The isolate was interpreted as susceptible, intermediate or resistant to a particular antibiotics by comparison with standard inhibition zones.

Results and Discussion

All samples were grown on Blood agar and MacConkey agar, they were sub-cultured on Blood agar, MacConkey agar and XLD agar. The isolates that were obtained from either MacConkey agar or XLD agar were identified according to observing characters, as the following:

Culturing on the three differential media:

Three differential media were used to isolate and identify *Proteus spp.* from other species of *Enterobacteriaceae* as a primary identification based on the most common characters [2], which are:

Blood agar was used to observe swarming phenomenon with no hemolytic activity.

MacConkey agar was used to observe the non- lactose fermenter isolates that appeared as pale, convex, and circular and smooth colonies, with special fish-like odour.

XLD agar was used to observe the colourless colonies of isolates.

Based on these common characters, 30 isolates of *Proteus* were submitted to biochemical tests

Microscopic examination

All isolates were appeared polymorphic Gram negative rods by using Gram stain [2, 3].

Properties of *Proteus* isolates

According to the biochemical properties, two species of *Proteus* were identified in this study; they were *P. mirabilis* and *P. vulgaris*.

Table-1 shows that these species were able to produce catalase, urease, gelatinase, but were not able to produce oxidase.As well as both isolates were able to utilize triple sugars and produced H_2S on TSI medium, and motile.

In addition, they showed positive results to methyl red test, negative results to Vogeus Proskauer test but no ability to utilize citrate as a carbon source. Remarkably, one test was carried out to distinguish between these isolates, it was indole production. *P. mirabilis* displayed negative result for indole production, while *P. vulgaris* showed positive result. These results were accorded to [1, 2, 11].

Table 1-Properties of *Proteus mirabilas* and *Proteus vulgaris*

Biochemical Tests			
Id	**Test**	***P. mirabilis***	***P. vulgaris***
1	Catalase production	+	+
2	Oxidase production	_	_
3	Urease production	+	+
4	Gelatinase production	+	+
5	Motility Test	+	+
6	(Triple sugar iron agar) TSI	Acidic slant / alkaline bottom, H_2S production	Acidic slant / alkaline bottom, H_2S production
7	Indole production	_	+
8	Methyl red test	+	+
9	Vogeus Proskauer tests	_	_
10	Citrate utilization	_	_
Bacteriological Tests			
1	Swarming on Blood agar	+	+
2	Haemolytic activity	Non haemolysis	Non haemolysis
3	lactose fermentation on MacConkey agar	Non lactose fermenter	Non lactose fermenter

(+): positive result; (-): negative result

Out of 152 clinical specimens of different infection sources, 30 specimens (19 %) were isolated and identified as *Proteus spp.* as in Figure-1. While other bacterial isolates belonged to other genus, so they were ignored. These result did not agree with [13, 14] who mentioned that *Proteus spp.* from clinical specimens represented (12.6%), and (8.4%) respectively.

The elevated percentage of local isolated may be because of the wide ability of Genus *Proteus* to invasive tissues and surfaces of instruments due to their virulence factors, in addition to wrong usage of Antibiotic drugs which generates increasing of *Proteus* infections and the contaminated urinary catheters or other indwelling devices used in an unclean environment of some hospitals.

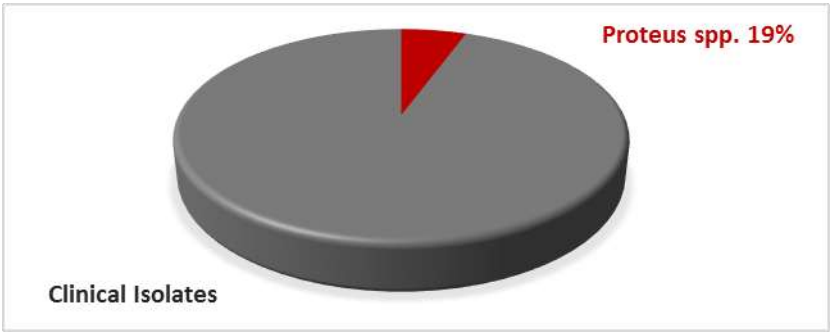

Figure 1-Percentage of *Proteus* spp. among clinical specimens

Twenty specimens (66.6%) of different clinical specimens were identified as *Proteus mirabilis*, and 10 specimens (33.3%) were identified as *Proteus vulgaris*, as in Figure-2. These results get an approach with (14) who mentioned that the percentage for *Proteus mirabilis* isolation was (61.5%) and for *Proteus vulgaris* was (30.5%). These results were not in agree with [15-17], that mentioned that the percentage isolation for *Proteus mirabilis* was (86.36%),(11.47%) and (85.7%) respectively, while for *Proteus vulgaris* was (13.64%),(3.27%) and (14.3%) respectively from UTI infections, but they showed the higher percentage of *Proteus mirabilis* infections than *Proteus vulgaris* which was (13.64%),(3.27%) and (14.3%) respectively from UTI infections, but they showed that *Proteus mirabilis* are more widespread than *Proteus vulgaris* in clinical infections, because *Proteus mirabilis* is a part of normal flora of human beings and other mammalians that leads to contamination of water or food with faces, while *Proteus vulgaris* and other species of *Proteus* are not part of normal flora of mammalians such as human, so they have less rate of infection [7].

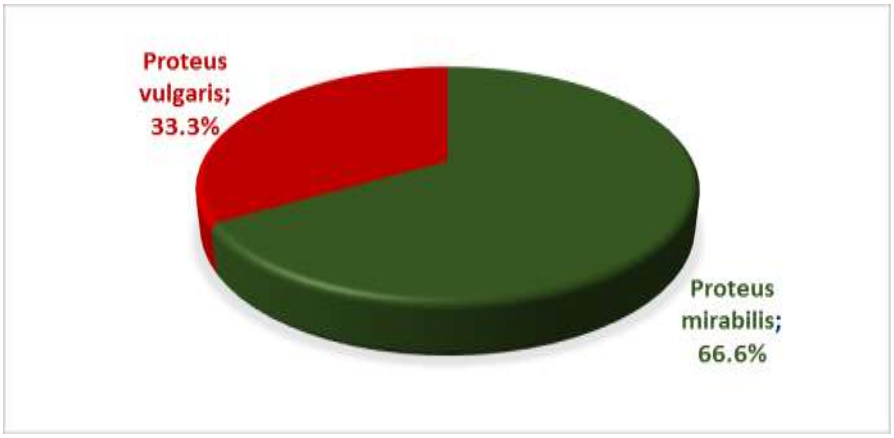

Figure 2- Percentage of *Proteus mirabilis* and *Proteus vulgaris* isolation

According to sex, out of 30 specimens that were identified as *Proteus spp.*, 17 (56%) specimens were from females and 13 (43%) specimens from males at different ages, as in Figure-3. These results agreed with [14], who mentioned that the percentage of isolation from female was (57%) and from male was (43%) but they not agree with [18] that showed (84.21%) percentage of isolation and (15.78%) isolation from males and females respectively.

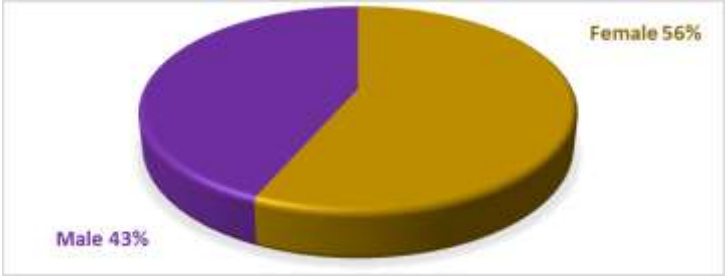

Figure 3- Percentage of *Proteus spp.* infections between males and females

Among 30 isolates of *Proteus spp.*, 18 isolates (60%) isolated from urinary tract infections (UTI), 7 isolates (23.3%) isolated from wounds and 5 isolates (16.6%) isolated from burns, as in Figure-4. The result of this study did not in agree with [14], who mentioned that the highest source of isolation was from wound infections (64.5 %).

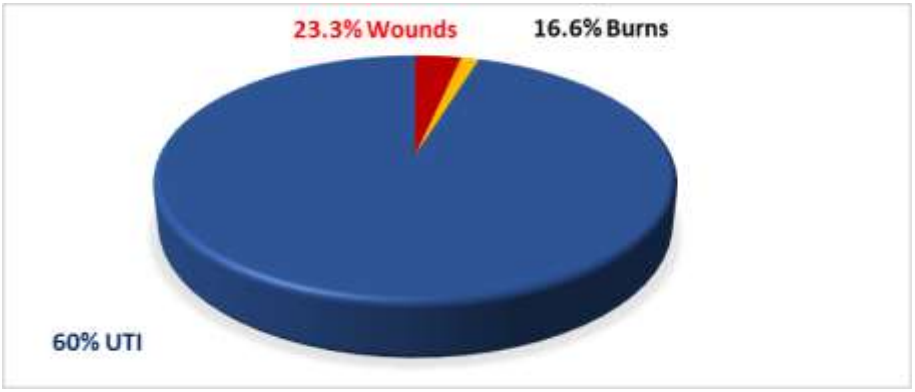

Figure 4- Percentage of *Proteus spp.* isolates from different sources of infection

Among 20 isolates of *Proteus mirabilis*, 13 isolates (65%) isolated were from urinary tract infections (UTI), the results of this study disagree with [15-17], who mentioned that the percentage isolation for *Proteus mirabilis* was (86.36%),(11.47%) and (85.7%) respectively, 4 isolates (20%) were from wounds specimens, which were in agree with (21), she mentioned that the percentage of *Proteus mirabilis* isolation from wounds was (19%), but not in agree with national studies such as: [1, 18, 19, 20] as they mentioned that the percentage of *P. mirabilis* isolation was (3.8%), (25.22%), (17.32%) and (5.26%), and finally 3 isolates (15%) were isolated from burns specimens, as in Figure-5.

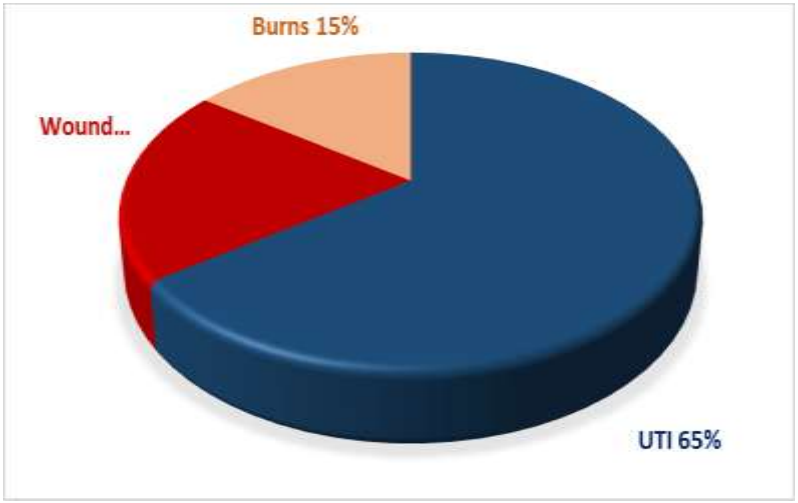

Figure 5-Percentage of *Proteus mirabilis* isolates from different sources of infections

The higher percentage of UTI infection than other infections may be due to the colonizing ability of *Proteus mirabilis* on the surface of urinary catheters or because it is part of normal flora of human gastrointestinal tract which increase the probability of UTI infection [8]. This confirm that *P. mirabilis* is the third most common (after *E. coli* and *Klebsiella pneumoniae*) cause of complicated UTI causing 12% of infections [4].

The susceptibility of 20 *P. mirabilis* isolates to 25 antibiotics (Ampicillin, Amoxicillin, Cloxacillin, Piperacillin, Cephlothin, Imipenem, Cephalexin, Cefoxitin, Cefotaxime, Ceftazidime, Cefixime, Cefepime, Chloramphenicol, Nalidixic acid, Tetracycline, Clindamycin, Rifampicin, Vancomycin, Clarithromycin, Erythromycin, Amikacin, Gentamicin, Kanamycin, Tobramycin, Streptomycin) was investigated by using Kirby-Bauer method.

Figure-6 indicated that *P. mirabilis* isolates had variable degrees of resistance towards different categories of antibiotics. It included clarithromycin, tetracyclin, clindamycin and erythromycin 100%, ampicillin, cloxacillin, cefixime and vancomycin 95% in spite of its action which is mostly on Gram positive bacteria [3]; cephalthin 90%, rifampicin 85%, amoxicillin 70%, cefotaxime, gentamycin and tobramycin 65%, piperacillin 60%, kanamycin 50%, cephalexin and nalidixic acid 45%, cefoxitin 35%, ceftazidime and streptomycin, amikacin 30%, cefepime and chloramphnicol 20%. Notable all *P. mirabilis* isolates were susceptible to Imipenem.

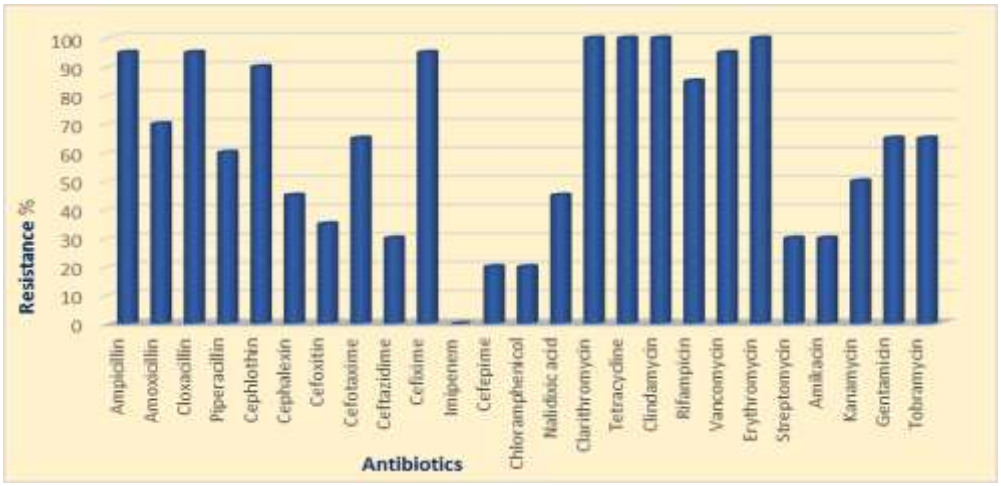

Figure 6-Susceptibility of *P. mirabilis* isolates to antibiotics

From the above results, it can be noticed that *P. mirabilis* isolates had moderate to low resistant to antibiotics, that inhibit protein synthesis, such as aminoglycosides (amikacin. streptomycin, gentamycin, tobramycin and kanamycin), Nalidixic acid, and chloramphenicaol, so as antibiotics that inhibit nucleic acids synthesis such as rifampicin, while all isolates showed resistance to erythromycin, clarithromycin, clindamycin, and tetracycline. Obviously, the results indicated that even Vancomycin is a protein synthesis inhibitor antibiotic and more remarkable with *Staphylococcus* but isolates recorded high resistance.

In addition, *P. mirabilis* isolates showed moderate to high resistance against antibiotics that inhibited the synthesis of cell wall such as pencillins with high sensitivity to imipenem. This was confirmed by the results of [22] that found a high susceptibility of Imipenem in different isolates of *Enterobacteracae*, and moderate to high resistant to some types of first generations of cephlaosporins in opposite to low resistancy to the most of third generation types used in this study in spite of increasing rate of resistance to cefaotaxime, but it is obvious that high activity of cefepime which is resistant to the fourth generation of cephalosporins.

Moreover, the results indicated that the resistance of *P .mirabilis* to some antibiotics is increased with prescription of years, because of the wrong and random use of these antibiotics and increasing the rate of *Proteus* infections. On the other hand, this bacteria had ability to produce β-lactamases, especially extended spectrum β-lactamases (ESBLs), as well as, their ability to transfer genetic elements carrying the genes of these enzymes, and number of mutations occur with these type of enzymes leading to increase resistance to antibiotic especially β-lactam, in addition to other mechanisms such as alteration the target site or alteration the access to the target site by modification of penicillin binding proteins (PBPs) [23, 24].

References:

1. Mordi, R. M. and Momoh, M. I. **2009**. Incidence of Proteus species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. *Afr. J. Biotechnol*.**,** 8 (5), pp:725-730, 6 March, **2009**.
2. Betty, A. F, Sahm, D. F. and Weissfeld, A. S. **2007**. *Bailey and Scott`s Diagnostic microbiology*. 12th.ed. Mosby, Inc., an affilinate of Elsevier Inc.

3. Brooks, G. F, Carroll, K. C., Butel, J. S. and Morse S. A. **2004**. *Jawetz, Melnick and adelberg`s Medical Microbiology*. 24th. ed. McGraw Hill companies, London.

4. Ro`zalski, A., Sidorczyk Z., and Kotelko A. **1997**. Potential virulence factors of *Proteus* Bacilli. *Microbiology and molecular biology reviews*, 61(1), pp: 65–89.

5. Guentzel M.N. **1996**. *Escherichia coli, Klebsiella, Enterobacter, Serratia, Citrobacter,* and *Proteus*. In: *Barron's Medical Microbiology*, Univ. of Texas Medical Branch at Galveston.

6. O'hara C. M., Brenner, F. W. and Miller, J. M. **2000**. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clinical microbiological reviews*.13 (4), pp: 534–546.

7. Wassif, C., Cheek, D. and Belas, A. **1995**. Molecular analysis of a metalloprotease from *Proteus mirabilis*. *J. of Bacteriology*, 177(20), pp: 5790–5798.

8. Mobley, H.L. and R. P. Hausinger.**1989**. Molecular characterization. Microbial ureases: significance, regulation, and molecular characterization. *Microbiology and molecular biology reviews*. 53(1), pp: 85-108.

9. Jacobsen S.M. and Shirtliff, M. E. **2011**. *Proteus mirabilis* biofilms and catheter-associated urinary tract infections. *Landes Bioscience*, 2(5), pp: 1-6.

10. Coker, C., Poore, C.A., Li, X. and Mobely, H.L. **2000**. Pathogenesis of *Proteus mirabilis* urinary tract infection. *J. Microbes infect*, 2 (12), pp: 505- 1497.

11. Harley, J. P. and Prescott, L. M. **2002**. *Laboratory exercises in Microbiolgy*, 5th. ed. McGraw Hill companies, London.

12. MacFaddin. **2000**. *Biochemical tests for identification of medical bacteria*. Lippincott Williams & Wilkins, USA.

13. Naz, S. A. and Rasool, S. A. **2013**. Isolation, production and characterization of bacteriocin produced by strains from indigenous environments. *Pak. J. Bot*, 45 (1), pp: 261-267.

14. Fegla, P.K; S. Y. Gbedema; S. N. A. Quay; Y. Adu-Sarkodie and C. O. Okrah. **2010**. Occurrence, species distribution and antibitics resistance of *Proteus* isolates: A case study at the Komfo Anokye teaching hospital in Ghana. *International Journal of pharma sciences and research,* Vol. 1 (9), pp: 347-352.

15. Al-Taai. Hadi Rahman Rasheed.**2005.** Bacteriological, biochemical and molecular study of *Proteus mirabilis* isolated from urinary tract infections in some hospitals of Baghdad city. PhD. thesis. Dept. of Biology. College of Sciences. AL-Mustansiriya University, Baghdad, Iraq.

16. Al-Kabby, Amer Saeed Ali. **2007**. Extraction and partial purification for fimbriae from *Proteus mirabilis* and study their role in adhesion to uroepithlial cells. Mc.S. Thesis. Department of Biology, College of Sciences, University of Baghdad. Baghdad, Iraq.

17. Al-Khateeb, Ahmed Fadhel kadhem. **2014**. Role of *Proteus mirabilis* DNA in comparison to *Candida albicans* DNA in rats' joints infection Mc.S. Thesis. Department of Biology, College of Sciences, University of Baghdad. Baghdad, Iraq.

18. Nagano, N., Shibata, N., Saitou, Y., Nagano, Y., and Arakawa, Y. **2003**. Nosocomial outbreak of infections by *Proteus mirabilis* that produce Extended –spectrum CTX-M-2 type β- lactamase. *Journal of clinical microbiology*, 41(12), pp: 5530-5536.

19. Nakano, R., Nakano, A., Abe, M., Inoue, M. and Okamoto, R. **2012**. Regional outbreak of CTX-M-2 β-lactamases= producing *Proteus mirabilis* in Japan. *Journal of Medical Microbiology*, (61), pp: 1727-1735.

20. Song, W., Kim, J., Bae, I. K., Jeong, S. H., Seo, Y. H., Shin, J. H., Jang, S. J., Uh, Y., Shin, J. H., Lee, M., and Lee, K. **2011**. Chromosome- encoded Amp C and CTX-M Extended spectrum β-lactamases in clinical isolates of *Proteus mirabilis* from Korea. *J. Antimicrobial agents and chemotherapy*, 55 (4), pp: 1414-1419.

21. Yasien. N., Najla`a. **2008**. Study the effect of ozone dissolved in water on bacteria *Proteus mirabilis* isolated from patients with different wounds and burns infection and on healing process of laborated animals infected with same bacteria. MS.c Thesis. Department of Biology, College of Sciences, University of Baghdad. Baghdad, Iraq.

22. Moosavian, M. and Deiham, B. **2012**. Distribution of TEM, SHV and CTX-M genes among ESBL- producing *Enterobacteraceae* isolates in Iran. *African journal of microbiology research*, 6 (26), pp: 5433-5439.

23. Rossolini, G.M., M. M.,`Andrea, D. and Mugnaioli, C. **2008**. The spread of CTX-M- type extended spectrum β-lactamases, *J. clin. Microbial infect*, 14, (1), pp: 33-41.
24. Cantón, R., Gonzalez-Alba, J.M. and Galan, J. C. **2012**. CTX-M enzymes. *Origin and Diffusion, Frontiers in Microbiology*, Vol. 3, article 110.