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Prevelance and antibiotic susceptibility patterns of *Pantoea* spp. isolated form clinical and environmental sources in Iraq

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Abstract : During the period from December 2014 to July 2015 , 40 isolates of *pantoea* spp. were isolated from 525 clinical and environmental sample from AL-Hakeem Hospital and AL-Sadder Medical City in AL-Najaf province. A total of 375 clinical samples from various sources included: Fistula-hemodialysis patients (n = 100), Stool (50), Urine (50), Ear-infection (25), Burn (100),Wound (50),While 150 hospital environmental specimen included system(50), bed (50) , earth (50). Then according to carbohydrate fermentation the 40 clinical and environmental isolates of *Pantoea* were distributed as follow *Pantoea agglomerans* 24 (60 %), 9(22.5 %) *Pantoea ananatis*, while 7 (17.5%) *Pantoea calida.*

The antibiotic resistance of *pantoea spp*. bacteria has profound clinical implications. Hence, this research was aim for the first time in Iraq to isolation and study prevelance of *pantoea spp*. From clinical and hospital environmental sources then determines their antimicrobial susceptibility patterns, the results revealed that *pantoea spp.* isolates associated with infection like hemodialysis inflammation (5), chronic diarrhea(5), UTI(3), otitis media (2), burn (10), wound (2), Also identified from 11 hospital environmental specimen included system (3) , bed (2), earth (6). At the same time the results found that *Pantoea spp*. were greatly resistant to antibiotics that most commonly used, so regard as multi-drug resistant(MDR), isolates exhibited high resistance to Amoxicillin and Gentamicin. While other antibiotics exhibited different activity against isolates. Furthermore, most isolates appeared low resistance to Meropenem and Amikacin while the isolates were sensitive to Imepinem which was the more effective on All isolates and the susceptible reached to (95%).

Conclusion The increase number of *Pantoea* species isolated from humans indicates that *Pantoea* is an opportunistic pathogen and consider as one of nosocomial infection in Iraq and These organisms are found to be resistance to the routinely used antibiotics. Appropriate antimicrobial drugs should be prescribed after detected antibiogram. The patients should also be prevent stop taking the drugs in the middle. This will help in minimising the complications, and help in preventing the emergence of resistant strains.

Key word : *Pantoea spp* , identification, clinical infection, antibiotic sensitivity.

Introduction

Pantoea spp. are gram-negative facultative anaerobic, rod-shaped Enterobacteriaceae, It moves by means of peritrichous flagellae[1, 2]. It was formerly known by several other names *Erwina* and *Enterobacter*[3] *Pantoea* spp. usually found in environmental housing for example soil and water, vegetables and seeds, also as plant pathogen[4, 5, 6, 7, 8, 9].

In recent years, studies have shown that *Pantoea* species cause a variety of diseases to humans increasingly[2]. A significant increase in nosocomial infections has been reported in different unites like Intensive Care, burn, haemodialysis and oncology department this lead to Sepsis as a complicated, multifactorial syndrome that can evolve into circumstances of various seriousness, portray as acute sepsis or septic shock[10]. Clinical markers are predominatingly unclear and, in a number of conditions, it may be hard or until not possible to detect the origin of the infection[11].

Pantoea spp. was observed during an outbreak caused by contaminated parenteral nutritional fluids in a Malaysian neonatal intensive care unit in 2006[12], total parenteral nutrition[13]. In India, Tiwaril and Beriha,[14] they reported that *P.agglomerans* as a cause of neonatal sepsis.

The evolution of antibiotic impedance in bacteria next foreword of antimicrobial agents has appear as an important failure therapy in infection anywhere in the world. It is a great challenge for clinician to treat these bacteria. In Iraq, there is no information available on the occurrence of antibiotics resistance in *Pantoea spp* In addition, there are very little information regarding the role of *Pantoea spp* in human bacterial infections. So, this study was conducted to isolate and identify *Pantoea* spp from different sources clinical and environmental by different methods and detect the susceptibility of bacteria to different type of antibiotic.

Materials and Methods

Isolation and Identification

A total of 525 clinical and environmental samples were collected during the study period from 1/12/2014 to 1/7/ 2015. These samples were collected from patients attending to Al-Sadder Medical City, Al-Hakeem General Hospital. 375 clinical samples were included Fistula-hemodialysis patients (100), Stool (50), urine-UTI (50), Ear infection (25), Burn (100) , Wound (50), while 150 environmental samples include system (50), bed (50), earth (50). All samples were cultured on the MacConkey agar plates, Blood agar plates and incubated at 37C˚ under aerobic condition for 18-24 hour, Gram's stain was used to examine the isolated bacteria for studying the microscopic properties such as gram reaction and shape .While Biochemical tests used according to MacFaddin,[15] in addition to use VITEK-2 system.

Antibiotic susceptibility testing

Antimicrobial susceptability testing was accomplished by the "Kirby-Bauer disc diffusion method using Mueller-Hinton agar" as recommended by Clinical Laboratory Standard Institute[16] . The antimicrobial agents tested and their corresponding concentrations were as follows: cephalothin (30µg), amikacin(10µg), norfloxacin(10µg), ceftriaxone(10µg), gentamicin(10µg), meropenem(10µg), amoxicillin(25µg), doxycycline (30µg), tobramycin (10µg), tetracycline(30µg), ciprofloxacin(5µg), cefotaxime(10µg), piperacillin(30µg), imipenem(10µg).

A small inoculum of *Pantoea* suspension (which prepared by inoculate 5 isolated grown on BHI agar to 5 ml of tryptic soy broth then incubated for 2hr. to produce a bacterial suspension of moderate turbidity that compared with turbidity of ready–made 0.5 McFaerland tube standard) was inoculated on Mueller-Hinton plates and antibiotic discs were placed on the plates, spacing them well to prevent the overlapping of inhibition zones. After incubating the inoculated plates aerobically at 37 C° for 18 to 24h, the susceptibility and resistance of the *Pantoea spp* isolates to each antimicrobial agent was measured and the results were interpreted in accordance with criteria provided.[16]

Results

The result clarified that the positive result for bacterial growth was 236 (44.95%) and 289 (55.05%) which were the rest of the samples consider negative results on Macconkey agar plate,. *Pantoea spp*. appeared as circles, middle in size, smooth, punctuate pink colonies with slow ferment lactose Fig.(1,A). While on blood agar *pantoea sp.* produce smooth, convex, rounded and usually non-hemolytic colonies, and non-pigmented or yellow, pale beige to pale reddish yellow colonies Fig. (1B).

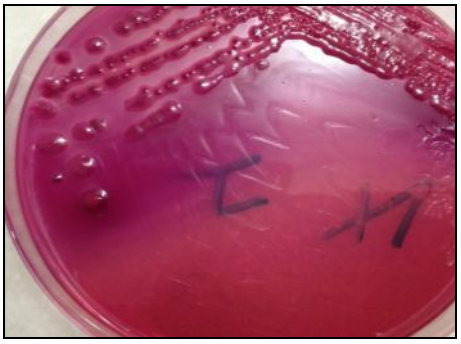
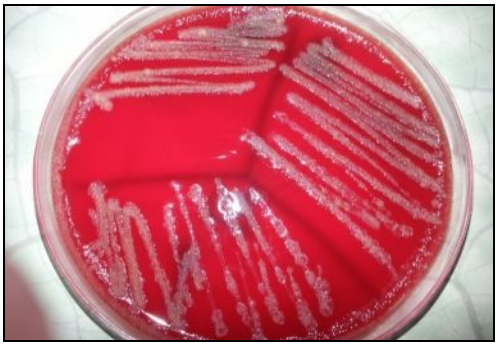
A  B 

Fig. (1): *Pantoea spp.* on culture media such as (A) MacConkey agar , (B) Blood agar .

Biochemical tests revealed that all isolates were positive results for catalase, citrate and motile, and negative results to produce indole and H_2S and produced other biochemical reactions as variable results (Table 1)

Table (1) Biochemical test for identification of *Pantoea* spp.

	Test	Result
1	Catalase	+
2	Oxidase	-
3	Hemolysis	-
4	Motility	+
5	urease	+/- (variable)
6	Indole	-/+
7	MR	-/+
8	VP	+/-
9	Citrate	+/-
10	TSI	A/A , K/A
11	Gas	+/-
12	H_2S	-

The final identification was performed with the automated VITEK-2 compact system using GN-ID cards which contained 47 biochemical tests and one negative control well. The results demonstrate that only Forty samples were obtained from clinical and environmental sources Back to *Pantoea* spp. (Table 2) with ID message confidence level ranging between very good to excellent (Probability percentage from 93 to 99). The *Pantoea spp*. have been identified to be 40 (16.94%) out of the total number of growth Gram-negative bacteria on MacConkey agar (n =236). All isolates were ferment Sucrose, Glucose, D-Xylose, Maltose, Galactose, L-Rhamnose and Cellobiose as shown in Table(2), But distinguish *P.agglomerans* from the other Species by it isn't ferment Lactose, D-Sorbitol and Lactulose. while distinguish *P.ananatis* from the other species by it is only species that ferment D-Arabitol and the *P. calida* Distinct from other species by ferment Raffinose. Data were taken from the following sources: Grimont and Grimont[17], and Brady *et al*[18].

Table (2): Forty samples were obtained from clinical and environmental sources

	No.(%)of *Pantoea* sp.	No. (%) of *Pantoea* species		
		P.agglomerans	***P.ananatis***	***P.calida***
Clinical sample				
Fistula-hemodialysis patients	5	3(60%)	0(0%)	2(40%)
Stool	5	3(60%)	2(20%)	0(0%)
Urine - UTI	3	2(66.7 %)	0(0%)	1(33.3)
Ear Infection	4	3(75%)	0(0%)	1(25%)
Burn	10	5(50%)	3(30%)	2(20%)
Wound –post operative	2	2(100%)	0(0%)	0(0%)
Hospital environmental sample				
System	3	2(66.7%)	1(33.3%)	0(0%)
Bed	2	1(50%)	1(50%)	0(0%)
Earth	6	3(50%)	2(33.33%)	1(16.67%)
Total	40	24(60%)	9(22.5%)	7(17.5%)

As shown in Table (3) the results of antibiotic sensitivity test for *pantoea spp.* Most bacterial isolates showed high resistance towards most widespread antibiotics, This called multidrug resistant(MDR). Hence, isolates exhibited high resistance to Amoxicillin(90%), Gentamicin (87.5%). While other antibiotics exhibited different resistant activity against isolates cephalothin (57.5%), cefotaxime (57.5%), ciprofloxacin (52.5%). Furthermore, most isolates appeared less resistance to Meropenem (12.5%) and Amikacin(25%) and 95% sensitive to Imipenem.

Table(3) : Antimicrobial susceptibility patterns of *Pantoea* spp. (N = 40)

Antibiotic	Resistant (%)	Intermediate (%)	Sensitive (%)
Amoxicillin	36(90)	4(10)	-
Gentamicin	35 (87.5)	5(12.5)	-
Tobramycin	22 (55)	10(25)	8(20)
Cefotaxime	23 (57.5)	11 (27.5)	6(15)
Ceftriaxone	20 (50)	11(27.5)	9 (22.5)
Ciprofloxacin	21(52.5)	12(30)	7(17.5)
Amikacin	10 (25)	10(25)	20 (50)
Piperacillin	20(50)	10(25)	10 (25)
Meropenem	2(5)	10(25)	28 (70)
Imipenem	-	2(5)	38 (95)
Tetracycline	21 (52.5)	12 (30)	7 (17.5)
Doxycycline	17(40)	14(35)	10 (25)
Cephalothin	23 (57.5)	6 (15)	11 (27.5)
Norfloxacin	22 (55)	9 (22.5)	9(22.5)
Total	19.64 (49.1)	9.3 (23.3)	11.07 (27.6)

Discussion

Generally little information are available about *Pantoea spp* especially in Iraq. *Pantoea spp* was considered as an uncommon cause of infections in human beings[19]. The results of morphology showed that *Pantoea* spp. appeared as convex mucoid, pink colonies on MacConcky because all these bacteria have the ability to lactose fermentation with production alkaline in media**.** In biochemical test the negative results to produce indole are because inability of the bacteria to produce tryptophanase that deamination of tryptophan to produce indole. The methyl red test was positive due to the production of the sufficient acid during the fermentation of glucose. Voges-Proskauer was positive because of the ability of bacteria to produce acetoin. A urease enzyme is released by microorganism breakdowns the C-N bond of amides to produce CO_2, ammonia

and H_2O, ammonia alteration pH of the medium that turned color to pink in a basic environment. All these results (morphology and biochemical) were identical with[15,20]. In study done by Sharma *et al*[21] they were isolated *Pantoea* from septic arthritis and have the same results in standard culture, biochemical test .

According to what we know, there is no previous study include the isolation of *Pantoea spp.* from clinical and environmental sources in Iraq to compare the present findings with it. These results in the same line with Boszczowski *et al,*[22] they recognized a nosocomial outbreak with *Pantoea agglomerans* in patients treated with hemodialysis. Laporte *et al*.,[23] stated that *Pantoea* is the causative agents in oteitis media infection. *P.agglomerans* causes keratitis[24], Endophthalmitis[25].

Studies by Cruz *et al*.,[1] indicated that *Pantoea* isolated from a variety of sites, involving the blood, arthritis, peritoneum, urinary tract and abscesses and Wound infection[26]. The results of this study nearly agreement with Nadarasah and Stavrinides,[27] they isolated *P. agglomerans* from both renal failure and urinary tract infections patients also they concluded that *Pantoea calida* was the common agents associated with UTI patients. At the same time study done in Taiwan by Cheng *et al*.,[28] who reported that *P. agglomerans* were one cause for Bacteremia. . This results online with study by Senanayake *et al.* [29] they found that *P.agglomerans* was responsible for the outbreak of blood stream infection occur in the NICU.

Fritz *et al*.,[30] they isolated *P. calida* from a cerebrospinal fluid (CSF) specimen and identified it as the cause of postsurgical meningitis. *P.ananatis* isolated from human wound in gorgia and considar as nosocomial infection as a result of using contaminated hospital material[31]. De Baere *et al*.,[32] isolated *p.ananatis* from septicemic patients following penetrating contaminated plant material.

A strain is considered a multidrug resistant (MDR) if an isolate is resistant to representatives of three or more classes of antibiotics[33]. So, some of these isolates are considered polydrug resistant this results agree with study done in Iran by Mardaneh *et al*.,[34] who explain that *P. agglomerans* strains isolated from consumed powdered infant formula milk (PIF) in NICU ward were multi-drug resistance.

The usage of antibiotics without antibiotics sensitivity testing, is the most important factor promoting the emergence of multi-drug resistance which lead to selection and dissemination of antibiotic resistant pathogens in clinical medicine[35]. Antibiotic resistance in bacteria may be an inherent trait of the organism (e.g. a particular type of cell wall structure) that renders it naturally resistant, or it may be acquired by Vertical Gene Transfer means of mutation in its own DNA or Horizontal Gene Transfer (acquisition of resistance-conferring DNA from another source where genetic material contained in small packets of DNA can be transferred between individual bacteria of the same species or even between different species[36].

In this study, most bacterial isolates were resistant to aminoglycoside antibiotics, this may be due to developed new resistant genes or by mutation through previous exposure to this group of antibiotics. Three methods of aminoglycoside resistance include: decrease uptake or reduced cell permeability, changes in the ribosomal binding locations, or making of "aminoglycoside modifying enzymes" [37].

The reason of β-lactam resistance isolates is may be because of the production of β-lactamase, which may be heritably localized on the chromosome or on a plasmid[38]. or lack of protein receptors on cell wall and alteration in their permeability to β-lactam antibiotics and preventing up taking of antibiotics by blocking the pores of outer membrane[39]. *Pantoea spp.* has ability to produce β-lactamase enzyme that has given the bacteria high resistance to several β-lactam antibiotics[40].

The resistance to third generation Cephalosporin's was caused mainly by mutations in the common TEM-1 and TEM-2 (two beta-lactamases, class A) enzymes and SHV-type enzymes which extended the hydrolytic spectrum of the enzymes to these antibiotics[41].

Generally, in this study, 95% of *Pantoea* spp. bacteria that isolated from different sources were susceptible to Imipenem. These results are correlated well with those obtained by Bayram *et al.* [42]. Imipenem are broad-spectrum carbapenems antibiotics. Beta-lactam rings of these antibiotics are resistant to hydrolysis by most β-lactamases and these antibiotic inhibits bacterial cell wall synthesis by binding to and inactivating PBPs [43] . Deletoile *et al.*,[44] they found that all strains of *P.agglomerans* were fully susceptible to broad-spectrum imipenem and in the same study they reports that *P. ananatis* was resistant to amoxicillin while study done by Liberto *et al*[10] they reported that *P.agglomerans* were resistance to ampicillin and cefazoline. Similar

observation for susaptability to meropenem were recorded by Tiwaril and Beriha,[14] who treated successfully neonatal sepsis with intravenous meropenem. Most bacteria have the ability to produce biofilm which consider important virulence factor that help in Bacterial persistence and prevent the effect of antibiotics and host immune defences[45] . Omran, 2016 illustrated that antibiotic resistance profile must be determined from time to time since the pathogenic bacteria change their ability in response to the antibiotics used to treatment the infections [46].

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