

Detection of some virulence factors in Pseudomonas aeruginosa associated with diarrhea in Kirkuk City

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ABSTRACT

Background: studies have shown that Pseudomonas aeruginosa can be a potential cause of antibiotic associated diarrhea. Pathogenesis based on endotoxin, exotoxins, and enzymes. Objectives: detection of some virulence factors produced by Pseudomonas aeruginosa isolated from faecal specimens in Kirkuk, Iraq.

Methods: The study included identification of Pseudomonas aeruginosa isolated from stool samples of children suffering from diarrhea, 518 samples collected from Azadi teaching hospital and Pediatric hospital in Kirkuk city, from February 2012 to June 2013. Various virulence factors including beta-lactamase, protease, lipase, lecithinase, deoxyribonuclease, gelatin liquefaction, haemolysin, congo red binding, urease, presence of capsule and betalactamase production were determined for Pseudomonas aeruginosa isolates.

Results: 35 Pseudomonas aeruginosa isolates were identified and the isolation percentage was 8.1% (only 433/518 give positive culture result for different bacteria). The distribution of virulence factors was different among the test isolates. All the isolates produce beta-lactamase but have different ability for binding to Congo red, gelatin liquefaction, lipase, protease, lecithinase, deoxyribonuclease, urease, capsule and haemolysin production.

Conclusion: Based on the findings of present study Pseudomonas aeruginosa isolated from diarrheal cases at least produced two virulent factors.

Keywords: Pseudomonas aeruginosa, diarrhoea, virulence factors.



دراسية بعض عوامل الضراوة لبكتريا Seeudomonas aeruginosa المسببة للإسبهال في محافظة كركوك المسببة للإسبهال في محافظة كركوك سهام شكور عبيد¹ ، عباس ياسين حسن² ¹ كلية التقنية كركوك / قسم التحليلات المرضية ¹ Suhama2011@yahoo.co.uk ² جامعة ديالي / كلية التربية للبنات تاريخ استلام البحث : 6 / 9 / 2014

الملخص

القيمة العلمية: بينت الدراسات بان جرثومة Pseudomonas aeruginosa قد يسبب الإسهال المرتبط باستعمال المضادات الحياتية. وتعتمد في امراضيتها على إنتاج السموم الداخلية، السموم الخارجية والأنزيمات.

الهدف: الكشف عن بعض عوامل الضراوة المنتجة من قبل جرثومة Pseudomonas aeruginosa المعزولة من عينات الغائط في مدينة كركوك-العراق.

طرق العمل: تضمن الدراسة عزل وتشخيص جرثومة الـ Pseudomonas aeruginosa من عينة الغائط لأطفال يعانون من الإسهال. تم تشخيص 35 عزلة من مجموع 518 عين غائط من مستشفى آزادي التعليمي ومستشفى الأطفال العام في مدينة كركوك، للفترة من شباط 2012 إلى حزيران 2013. تم تقديرا لعديد من عوامل الضراوة وهى إنتاج أنزيم البيتا لاكتاميز، البروتيز، اللايبيز، الليسيثينيز، الأنزيم المحلل للـ DNA، اليوريز، تحلل الجيلاتين، إنتاج الكبسول، إنتاج الهيمولايسين، الإختراقية لخلايا العائل (الارتباط بصبغة الكونغو الأحمر) لـ 35 عزلة

. Pseudomonas aeruginosa

النتائج: كانت نسبة العزل 8.1% (فقط 433 عينة أعطت نتيجة زرع موجبة لمختلف أنواع البكتريا). وقد أظهرت الدراسة بان جميع العزلات منتجة لأنزيم البيتا لاكتاميز ولكنها متفاوتة في إنتاجه للعوامل الأخرى.

الاستنتاج: استنادا إلى نتائج الدراسة الحالية ان جرثومة Pseudomonas aeruginosa تنتج على الأقل عاملين من عوامل الضراوة.

الكلمات الدالة : Pseudomonas aeruginosa، المقاومة للمضادات الحياتية.



1.INTRODUCTION

P. aeruginosa is widely distributed in nature. It can colonize normal humans, in whom it is a saprophyte. It causes disease in humans with abnormal host defenses [1]. P. aeruginosa produces a number of virulence factors which after colonization can cause extensive tissue damage, bloodstream invasion, and dissemination [2], also can cause the infections of gastrointestinal tracts with diarrhea in infants, and septicemia was manifested as necrotizing bowel lesions with a history of diarrhea [3]. P. aeruginosa can cause disease in any part of the gastrointestinal tract (GIT) from the oropharynx to the rectum, as in other forms of P. aeruginosa disease, those involving the gastrointestinal tract occur primarily in immunocompromised individuals. The bacterium has been implicated in perirectal infections, pediatric diarrhea, typical gastroenteritis, and necrotizing enterocolitis. The (GIT) is also an important portal of entry in septicemia caused by this bacterium [4]. However studies have shown that P. aeruginosa can be a potential cause of antibiotic associated diarrhea⁽³⁾. Pathogenesis based on endotoxin, exotoxins, and enzymes; its endotoxin, like that of other gram-negative bacteria, causes the symptoms of sepsis and septic shock [5]. Most strains of P. aeruginosa produce two exotoxins, the importance of these putative virulence factors depends upon the site and nature of infection [6], compared with other gram-negative organisms, P. aeruginosa is typical because it secretes a large number of proteins during growth [7], and utilizes a number of distinct pathways to secrete proteins that play various roles during infection [8]. Accordingly, the aim of this work was to detect the production of some virulence factors (gelatinase, protease, lecithinase, urease, hemolysin, and congo red binding) isolated from children suffering from diarrhea.

2.MATERIALS AND METHODS

2.1.Sample collection

The study was carried out on children (out and inpatient suffering from diarrhoea) attending Azadi teaching hospital and Pediatric hospital in Kirkuk city, from February 2012 to June 2013, 518 Stool specimens were collected in disposable, clean screw-capped, used for this purpose. All the specimens were processed immediately or used Carry Blair transport media if delayed for 1-2 hours after their collection and then cultured [9].



2.2.Bacterial isolation and identification

Collected samples were cultured onto nutrient, blood and MacConkey's agar for primary isolation. Non-lactose fermented colonies were selected and by using biochemical tests and API 20E System (BioMérieux/France) for identifying P. aeruginosa [10].

2.3.Detection of some virulence factors

- 1. Gelatin liquefaction test [11].
- 2. Hemolysin production [11].
- 3. Capsule production [11].
- 4. Binding to Congo red [11].
- 5. Protease production [11].
- **6.** DNase production [11].
- 7. Urease activity [11].
- **8.** Lecithinase production [11].
- 9. Lipase production (Tween 80 hydrolysis test) [10].
- **10.**β-lactamase production [11].

3. RESULTS AND DISCUSSION

3.1.Isolation and identification

The results of identification were showed that among the 433 sample that gave positive culture, 35 (8.1%) P. aeruginosa Table.(1) isolated from children with diarrhea, and the species isolated in male more than female and among (2-12 months) age group more than other age groups Table.(1) and Table.(2). The increased incidence in this age is most likely the result of greater exposure to enteropathogens (as the use of solid food starts at this age), plus the presence of susceptible host [12] due to the immaturity of the immune system of the child, as well as the decline of the passive immunity after its 6 months of age [13]. We agree with some authors that the higher rate of isolation of P. aeruginosa in children suffering from diarrhea may contribute to contamination drinking tap water in Kirkuk province, as they reported, and this is considered as a public health hazard for human beings [14, 15].



Table.(1): Distribution of P. aeruginosa according to patient's sex.

Gender Isolates	Male	Female	Total	
P. aeruginosa	20 (57.14%)	15 (42.56%)	35(100%)	

Table.(2): Distribution of *P. aeruginosa* according to patient's age groups.

Isolates	Age group/year				Total
	0.2-1	1.1-5	5.1-10	10.1-13	
P. aeruginosa	16	15	3	1	35
	(45.71%)	(42.86%)	(8.57%)	(2.86%)	(100%)

3.2. Virulence factors of P. aeruginosa

Many evidence indicated that markedly different microbial pathogens use common strategies to cause infection and disease [16]. Many diverse bacterial pathogens share common mechanisms in terms of their abilities to adhere, invade, and cause damage to host cells and tissues [17], as well as to survive host defenses and establish infection. Many of these infections appeared to be related to the acquisition of large blocks of virulence genes from a common microbial ancestor, which can be disseminated to other bacteria [18]. In the present study, a total of 35 P. aeruginosa were isolated from children suffering from diarrhea.

1.Detection of β-Lactamase Enzymes Production

The production of β -lactamase enzymes by isolates were determined by rapid iodometric method. All the isolates 35/35 (100%) were β -lactamase producer Table.(3). This result was in accordance with the study of Al- Al-Mashhadani, (2004) in which all P. aeruginosa isolates recovered from different samples were (100%) β -lactamase producers [19]. It was found that the major mechanism of resistance in gram negative bacteria causing clinically significant infection is the expression of β -Lactamases, of which there are several classes including plasmid encoded and chromosomally encoded enzymes [20].

2. Gelatin liquefaction

In the nutrient gelatin media, 34/35 (97.14%) of P. aeruginosa strains demonstrate gelatin hydrolysis activity Table.(3), which is in agreement with many researchers that reported high ability of P. aeruginosa to produce this enzyme, and it has an important in pathogenicity [21, 22, 23].



3.Hemolysin production

In the blood plate method, 16/35 (45.71%) of P. aeruginosa strains demonstrate betahemolytic activity Table.(3), which is in agreement with Al-Mashhadani, (2004) who stated that hemolysin is produced by (66.66%) P. aeruginosa isolated from rectal swab and stool samples [19]. Another study detected combinations of various pathogenicity factors, adhesiveness and hemolytic activity were shown by 13.5 per cent of the strains, while production of enterotoxin and hemolysin together was measured in 16.9 per cent of the strains. Adhesive activity, enterotoxigenicity and hemolysin production were observed in 6.7 per cent of the strains [24]. Hemolysin production is associated with necrotoxicity and cytotoxicity of cell and can destroy the erythrocyte to extract iron from them [25]. Many hemolysins probably form pores in the plasma membrane of erythrocytes, so hemoglobin and/or iron are released [26]. In a normal clinical situation all the virulence factors in conjunction may be deciding the outcome of an infection and hence all should be considered. Besides considering levels of all extracellular enzymes, high levels of haemolysin production in vitro may be used as surrogate information for pyelonephritic potential of P. aeruginosa [27].

4.Capsule

The results in present study indicated that 15/35 (42.86%) isolates were able to produce a capsule Table.(3) .The result unagreed with the results of Al- Al-Mashhadani, (2004) who reported P. aeruginosa that isolated from stool and rectum swab were non-capsulated [19], while relatively agreed with the results of Ismail, (2006) whom reported that 22/65 (33.9%) of P. aeruginosa isolated from stool of children suffering from diarrhea were capsule procure [14]. Capsule is often produced only under specific growth conditions, even though not essential for life, capsules probably help bacteria to survive in nature and help many pathogenic and normal flora bacteria to initially resist phagocytosis by the host's phagocytic cells and prevent killing by bactericidal serum factors. As well as in soil and water, prevent bacteria from being engulfed by protozoans, and also help many bacteria to adhere to surfaces and thus resist flushing [28].



5.Lipase

Concerning the production of lipase enzyme 5(14.29%) isolates of P. aeruginosa were positive for lipase production. The result unagreed with the results of Al-Mashhadani, (2004) who reported P. aeruginosa that isolated from stool and rectum swab were negative for lipase production [19]. The major factor for the expression of lipase activity has always been carbon, since lipases are inducible enzymes [29], and are thus generally produced in the presence of a lipid source such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, tweens, bile salts and glycerol, However, their production is significantly influenced by other carbon sources such as sugars, polysaccharides, whey and other complex sources. Among the different carbon sources used [30], olive oil was found to be the most suitable source. Most published experimental data have shown that lipid carbon sources (especially natural oils) stimulate lipase production [31,32], and olive oil and peptone were the most suitable substrate for maximum lipase production by P. aeruginosa [30].

6.Extracellular protease

Concerning the production of protease enzyme 5(14.29%) isolates of P. aeruginosa were positive for protease production. The result unagreed with the results of Al-Mashhadani, (2004) who reported that all P. aeruginosa that isolated from all clinical specimens all (100%) protease producers included stool and rectum swab [19]. Protease production by P. aeruginosa differs according to the source of specimens, type of colony and severity of diseases [21, 22], increased in protease production increased invasiveness and pathogenicity ability in P. aeruginosa.

7.lecithinase

Concerning the production of lecithinase enzyme 4(11.43%) isolates of P. aeruginosa were positive for lecithinase production. In a study conducted by Al-Mashhadani, (2004) revealed that lecithinase production by P. aeruginosa differ according to the source of samples [19]. The importance of Lecithinase is destroying red blood cells and other tissue cells. It is active in phosphatidylserine and phosphatidylcholine degradation. Lecithinase hydrolyzes lecithin which is a lipid component of eukaryotic membrane thereby this enzyme destroys the integrity of the cytoplasmic membranes of many cells. The two enzymes (lecithinase and Hemolysin) have a synergistic effect on the ability of the organism to invade host tissues [14]



8.Urease activity

Concerning the production of urease enzymes 4(11.43%) isolates of P. aeruginosa were positive. Mobley et al., (1995) reported that the production of P. aeruginosa virulence factors are depending on the site of infection and were induced by the presence of the substrate of the certain factor in this site (the composition of the surrounding area) [33]. Mclean et al., found that urease provided a suitable condition for bacterial survival in the site of infection through changing the pH and the removal of the toxic effect of urea by converting it to CO_2 and NH_3 [34]. As a result of ammonia production, an increase in local pH causes precipitation of normally soluble calcium and magnesium ions. These salt crystals can grow to remarkable size to produce bladder and kidney stones [35].

9.Invasiveness (binding to Congo red)

Congo red (CR) dye agar test was first used by Surgalla and Beasly for differentiation of virulent and a virulent Yersinia pestis [36]. Subsequently, it was used other researchers as phenotypic marker to differentiated invasive and non- invasive E. coli and Shigella spp. [37]. In the present study, the results showed that from 35 P. aeruginosa 4(11.43%) gave positive (red colony) Table.(3).

10.DNase production

Concerning the production DNase enzyme all P. aeruginosa isolates were negative for this test, which is in agreement with Janda and Bottone, (1981) and Al-Mashhadani, (2004) whom stated that P. aeruginosa isolates that isolated from stool and rectal swab did not produce DNase enzyme, and they reported that the production of DNase are depending on the site of infection [14, 18].



Isolates	Pseudomonas aeruginosa (n=35)		
Virulence factors	no	%	
β-lactamase production	35	100%	
Gelatin liquefaction	34	97.14%	
Haemolysin	16	45.71%	
Capsule	15	42.86%	
Lipase	5	14.29%	
Protease	5	14.29%	
Lecithinase	4	11.43%	
Urease	4	11.43%	
Congo red binding	4	11.43%	
Deoxyribonuclease	0	0.00%	

Table.(3):	Virulence	factors	possessed	by P.	aeruginosa	(n=94)
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