Virulence Factors of Proteus Mirabilis Isolated From Patients Otitis Media in Baquba And it's Peripheries

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Abstract

Background: Otitis media is one of the well known diseases that infect children and adult. The severity of infection caused by the members of the genus Proteus depends mainly on the availability of virulence and pathogenicity factors.

Objectives: to detect and evaluate the presence of virulence factors in local isolates of Proteus species causing otitis media.

Patients and methods: Two hundred and seventy ear swabs were collected from patients with otitis media attended Baquba General Hospital for the period from. Swab were cultured on blood, MacConkey and chocolate agar plates and incubated at 370C for 24 hrs. Chocolate agar plates were incubated under 10% CO2 (Candle jar). Suspected colonies of proteus species were isolated and subcultured on MacConkey agar. The identification of bacterial isolate was performed according to the standard biological and biochemical methods. Virulence factors including; urease, β-Lactamase and extended spectrum β-Lactamase (ESBLs), Human and ovine blood agglutination and adhesion to epithelial cells, hemolysin, esterase and proteolytic enzymes were detected following standard biochemical methods. Biofilm formation was detected by enzyme-linked immunosorbant assay.

Results: Out of 270 ear swabs, 240 (88.9%) were positive for bacterial growth and of these 35 (12.9%) were identified as of Proteus species. Following the standard biochemical and enzymes tests, 7 (2.6%) was found to be Proteus vulgaris and 28 (10.4%) were Protues mirabilis.

Conclusion: P.mirabilis appeared as one of the causative agent of otitis media in patients from Diyala province, and most isolates have multiple virulence factors that may increase their infectivity and worsen the clinical picture of the disease.

Keywords: Otitis media, Proteus mirabilis, Virulence factors

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Introduction

Proteus mirabilis a member of the Proteus genus that belongs to the family Enterobacteriacae, is a gram negative short rods, mobile and non-spore former bacterium. Members of the Proteus genus are saprophytic normal flora or opportunistic pathogens causing many infections when they colonize areas out of their natural habitat [1]. Proteus mirabilis is one of the most effective agents that causing otitis media [2]. Otitis media is one of the well known diseases that infect children and adult, which is a part of upper respiratory tract infections that include sinusitis and pharyngitis [3]. The severity of any infection caused by the members of the genus Proteus depends mainly on the availability of virulence factors that may include β-Lactamase, extended spectrum β-lactamases, Protease, Urease and hemolysin production [4-7]. Other factors like swarming motility, adhesion and biofilm formation are also important [8,9]. All these factors collectively or separately play important roles in pathogenicity and pathogenesis of disease [10]. Accordingly, this study was performed to detect and evaluate the presence of virulence factors in local isolates of Proteus.

Patients and Methods

Two hundred and seventy ear swabs were collected from patients with otitis media attended Baquba General Hospital for the period from. Swab were cultured on blood, MacConkey and chocolate agar plates and incubated at 370C for 24 hrs. Chocolate agar plates were incubated under 10% CO2 (Candle jar). Suspected colonies of proteus species were isolated and subcultured on MacConkey agar. The identification of bacterial isolate was performed according to the standard biological and biochemical methods [11]. Twenty four Proteus isolates were used for detection of virulence factors. The standard methods that used for the detection of urease [12], β-Lactamase and extended spectrum β-Lactamase (ESBLs) [13], Human and ovine blood agglutination and adhesion to epithelial cells [7], and detection of hemolysin [14] were followed. Furthermore, the same isolates were subjected to, biofilm formation test by enzyme-linked immunosorbant assay according to the method described by (sandoe et al., 2003)[15], esterase production test and detection of proteolytic enzymes [16].

Results

Out of 270 ear swabs, 240 (88.9%) were positive for bacterial growth and of these 35 (12.9%) were identified as of Proteus species, while other 205(75.9%) yielded bacterial growth other than proteus. Following the standard biochemical and enzymes tests, 7 (2.6%) was found to be Proteus vulgaris and 28 (10.4%) were Protues mirabilis, table (1).

Table 1: The results of ear swabs cultivation for bacterial growth.

Results of Cultivation		No. of Samples	%
Negative for Bacterial Growth		30	11.1
Bacterial Growth other than *Proteus*		205	57.9
Proteus Positive Growth	*P. vulgaris*	7	2.6
	P. mirabilis	28	10.4
Total number		270	100

The investigation of 24 Proteus isolates for 10 virulence factors showed that not all the isolates had the same number of the factors. No bacterial isolate appeared negative for urease while only one isolate appeared negative for hemagglutination of human and ovine blood. The ability to produce biofilm appeared in 22 (91.7%) of the above mentioned isolates. Furthermore, 21 (87.5%) of the isolates showed the ability to adhere to human epithelial cells in vitro. The ability for lysis sheep RBCs appeared in 18 (75%) isolates, while the ability for lysis of human RBCs appeared in 16 (66.7%) isolates. The results also showed less than half number (45.8%) of the isolates appeared positive for β- Lactamase and protease. Extended spectrum β-Lactamase produced by 8 (33.3%) isolates only. Esterase enzyme was detected in18 (75%) isolates, table(2).

Table 2: The number of *Proteus* isolates positive for particular virulence factor.

Virulence Factors	Positive		Negative	
	No.	%	No.	%
Urease production	24	100	0	0
Agglutination of RBCs	23	95.8	1	4.2
Biofilm Formation	22	91.7	2	8.3
Adhesion to Epithelial cells	21	87.5	3	12.5
Esterase Production.	18	75	6	25
Lysis of Ovine RBCs	18	75	6	25
Esterase production	18	75	6	25
Lysis of Human RBCs	16	66.7	8	33.3
β-Lactamase production	11	45.8	13	54.2
Protease production	11	45.8	13	54.2
Extended spectrum β-Lactamase	8	33.3	16	66.7

The virulence score of each of 24 isolates was also estimated. One isolate only had three factors, urase, esterase and adhesion factor. Two isolates produced five similar factors (urease, β-lactamase, hemagglutination, biofilm and adhesion factor). The ability to produce urease, biofilm, esterase, adhesion factor, and lysis of human RBCs appeared in 6 isolates only. Seven isolates lost the ability to produce protease, β-lactamase and extended β-lactamase. The esterase enzyme and the ability for lysis of human RBCs were not detected in five isolates. Furthermore, four isolates showed no esterase enzyme; however, only one isolate appeared to have the 10 above mentioned factors, table (3).

Table 3: Virulence factors score of *Proteus isolates.*

No. of the virulence factors	Number of isolates	%	Cumulative percentage
3	1	4.2	4.2
5	2	8.3	12.5
6	3	12.5	25
7	8	33.3	58.3
8	5	20.8	79.1
9	4	16.7	95.8
10	1	4.2	100
Total	24	100	

The study of hemolysin types of the isolates showed that 8 (33.3%) and 6 (25%) of them were unable to lyse human and sheep RBCs respectively. Those producing Alpha type of hemolysin for human and sheep RBCs appeared in 5 (20.8%) and 6 (25%) isolates respectively. Furthermore, 11 (45.8%) isolates produced Beta type of hemolysin of human RBCs and 12(50%) produced Beta type of hemolysis of sheep RBCs.

Table 4: Type of hemolysin produced by Proteus isolates.

Type of hemolysin	Hemolysis of human RBCs		Hemolysis of sheep RBCs	
	Number	%	Number	%
No hemolysis	8	33.3	6	25
α-hemolysis	5	20.8	6	25
β-hemolysis	11	45.8	12	50
Total	24	100	24	100

Discussion

Acute otitis media usually arises as a complication of a preceding viral upper respiratory infection. Accumulation of serous effusion in the middle ear provides a fertile media for microbial growth and rapid middle ear infection develops which is most commonly caused by viral, bacterial, or fungal pathogens [17]. Proteus species are among frequently isolated bacteria from such conditions aided by its various virulence factors [1,2]. As the present study aimed to investigate the isolation rate of proteus species from ear discharge of patients with otitis media and to explore their relevant virulence factors, the results found that 12.9% of the ear swabs culture was positive for proteus species. Actually, these results were almost similar to that reported by another worker [18-20]. On the other hand, the negative cultures may be attributed to infection by fungi, viruses or to the presence of anaerobic or fastidious bacteria [21,22]. Regarding the virulence factors, the results showed that all Proteus isolates were urease positive. These results are in agreement with those reported by other workers [10,14,23]. Likewise, 95.5% of P.mirabilis isolates agglutinated human RBCs, and this is again consistent with previous reports [5,14].

The biofilm formation was found in 91.7% of our isolates, and this come in agreement with the findings reported by many authors [9,24]. It is worth to mention that the formation of biofilm was increased in alkaline medium that mediated by urease enzyme produced by the Proteus [25]. Other studies exposed the role of fimbriae in aggregation of bacteria and formation of biofilm that increased the

infectivity of the organism [26]. It has been documented that the biofilm played an important role in antibiotic resistance of the microorganism[27].

These findings strongly supported the present results concerning the ability of Proteus isolates for formation of urease, biofilm, adhesion factor and agglutinate human blood. High ability of P.mirabilis for adhesion to epithelial cells was documented and attributed to the presence of cilia and pili of microorganism, and also to the ability of biofilm formation [4,7,8,10].

Several studies including the present one had drawn the attention to the ability of P.mirabilis to hemolyze sheep RBCs. Although these studies had obtained different rates of hemolysis, they all point out to the remarkable ability of this bacteria to hemolyze sheep RBCs [5,14,28]. The present results also revealed that the type of hemolysin produced by Proteus species, weather it is alpha or beta type, had almost equal hemolytic activity against human or sheep RBCs [14,29].

In the present study 45% of proteus isolates were β-lactamase positive. Of note, studies conducted in this field had yielded different positivity rates [30.31]. Interestingly, it has been demonstrated that β-lactamase positive proteus isolates correlates better with biofilm formation, but not with cellular adhesion ability [4]. However, it has been documented that the β-lactamase positivity rate was influenced by region and the sensitivity of detection method [32]. On the other hand, the Extended spectrum β-lactamases which are plasmid-mediated β-lactamases capable of efficiently hydrolyzing penicillins, narrow spectrum cephalosporins, many extended-spectrum cephalosporins, the oxyimino group containing cephalosporins (cefotaxime, ceftazidime), and monobactams (aztreonam) [33]. ESBLs were detected in 33.3% of the present isolates. Again previous studies had reported different positivity rates which were relevant to bacterial species, clinical specimens and sensitivity of detection system [4,6,13,31,34]. Furthermore, it has been reported that the prevalence of ESBLs in P. mirabilis was increased from 0.5% in 2005 to 20.9% by 2008 [35]. The ability of proteus species to produce protease enzyme seems to be varied. The present study found 45.8% isolates were positive to such enzyme. Senior et al. (1991)[36] found that 64% of P. mirabilis isolated from UTIs protease enzyme producer, and in another study he reported that 94% of P. mitabilis, 71% of P.vulgaris and all P. penneri from diverse clinical specimens were protease positive [16].

In a final conclusion, P.mirabilis appeared as one of the main causative agent of otitis media in patients from Diyala province. Most of these isolates had multiple virulence factors that increase their infectivity and worsen the clinical picture of the disease and may interfere with the efficiency of antimicrobial therapy. Accordingly, molecular studies to point out the exact role of virulence factors in the infectivity of P.mirabilis and its resistance to antimicrobials are recommended.

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