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Original Research Article

Virulence Factors of Proteus Species Causing Catheter-Associated Urinary Tract Infection

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One of the most common hospital-acquired infections (HAIs) is catheter-associated urinary tract infection (CAUTI). *Proteus species (spp)* are the primary infectious agent in patients with long-term indwelling urinary catheters. We aim to study some virulence factors of *Proteus spp* isolated from CAUTIs; three types of motility: swarming (SW), swimming (SM) and twitching (TW), urease production, biofilm formation and swarming over three different materials of urinary catheters (latex, silicon and silicon coated latex), also, *Proteus* isolates were tested for their sensitivity to different classes of antibiotics by disc diffusion method. This study was carried on 110 urine samples from CAUTI cases. *Proteus* isolates were (10/110 isolates, 12.5%). All of them were urease producers, strong biofilm former and had the ability to twitch, swim and swarm all over the plates except one strain. Four strains could bridge over latex catheters while two strains could bridge silicon catheters and silicon coated latex catheters. Seven strains were multidrug resistant (MDR) and two strains were extremely drug resistant (XDR) while only one strain was sensitive, so *Proteus* isolates from CAUTI are very virulent, so insert urinary catheter only if indicated and if you suspect long duration, use silicon better than latex.

Keywords: CAUTIs, *Proteus*, virulence factors, Catheter, Urinary tract infection.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common hospital-acquired infections (HAIs); the indwelling urethral catheters are responsible for 70%–80% of these infections (Saint and Chenoweth, 2003, Weber et al., 2011). The risk of infection depends on the duration of catheterization mainly and other factors as improper catheter management (Meddings et al., 2010). The large numbers of catheter-associated urinary tract infections (CAUTIs) are associated with the biofilm formation (Stickler, 2008). One of the most important pathogens in this regard is *Proteus mirabilis* (*P. mirabilis*) (Hola et al., 2012). *Proteus* is one of the commonly implicated pathogens in HAIs as well as community-acquired infections (CAIs) (Kwil et al., 2013). The three *Proteus spp* that are associated with UTIs, are *P. mirabilis, P. vulgaris,* and *P. penneri* (Senior and Leslie, 1986).

Pathogenic bacteria have developed numerous virulence factors to adapt to their host environment, so studying the main *Proteus* virulence factors would increase our understanding of how a microorganism from normal flora infects and colonizes other sites and establishes infection (Coker et al., 2000). Accordingly, the aim of this work was to study some virulence factors produced by *Proteus spp* isolated from patients who had indwelling urethral catheters and developed CAUTI according to the recent definitions of the center of disease prevention and control (CDC).

MATERIALS AND METHODS

Study type

This study is a descriptive cross-sectional one.

Study Area

This study was carried out in Tanta University Hospitals (TUHs), El-Gharbia, Egypt. This hospital is located in El Delta in the northern part of Egypt.

Study duration

This study was carried out during the period from January 2015 until the end of October 2015.

Study Population

A total of 110 patients within the age range of 1 to 95 years were included in the study. The inpatients (n=89) were

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selected from the pediatric intensive care unit (ICU) (n=6) and adult ICUs (n=73) and the outpatients (n=31) were selected from Urology department clinics in Tanta University. All patients recruited for this study fulfilled the inclusion criteria of CAUTI (CDC, 2015) and exclusion criteria were catheterized inpatients or outpatients for less than 2 days, suprapubic catheterized patients or patient with external catheter "condom catheter".

Definitions

CAUTI is defined as a UTI where an indwelling urinary catheter was in place for >2 calendar days on the date of event, with the day of device placement being Day 1, and an indwelling urinary catheter was in place on the date of event or the day before(CDC,2015).

Multidrug resistant (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

Extensively drug resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two antimicrobial categories) (Magiorakos et al., 2012).

Ethical Consideration

Ethical approval was obtained from the ethics committee of Quality Assurance Unit, Faculty of Medicine, Tanta University, Egypt. The participants (subjects) were briefed on the aim and procedure of the study. Verbal and written consents were obtained from each of the participants.

Sample Collection and Handling

Urine specimens were collected from 110 patients who had indwelling latex urinary catheters under complete aseptic technique (Hand hygiene with alcohol hand rub, clean gloves, sterile syringe and alcohol pads for disinfection of syringe insertion site for sampling and maintain a closed drainage system) then transferred immediately to the laboratory of Microbiology and Immunology Department in Faculty of Medicine, Tanta University where urine specimens were transferred to sterile, dry, leak-proof, disposable, labeled universal containers (CLSI, 2001).

Urine samples were cultured by sterile calibrated plastic loops on cysteine lactose electrolyte deficient (CLED) agar (Oxoid, UK), aerobically at 37 °c for 24 hrs, on the following day, CLED plates were examined and subcultures were done for cases with significant viable count only (the significant colony count is a pure growth of $\geq 10^5$ CFU/mL in case of bacteriuria and $\geq 10^4$ CFU/mL in case of candiduria)(Deorukhkar and Saini, 2016).The subcultures were done on nutrient, blood, MacConkey's agars (Oxoid,UK); all plates were incubated aerobically at 37 °c for 24 hrs. The produced colonies were identified by the traditional methods including microscopic examination, culture characteristics and biochemical reactions for detection of the causative organisms (Parija, 2012, Tille, 2013).

Proteus spp identification

On blood and nutrient agars, we checked the presence of swarming phenomenon and fishy smell of *Proteus*. According to (Tille, 2013, Cheesbrough, 2006). *Proteus spp* were identified by the following biochemical reactions; glucose and sucrose fermentation with or without production of gas and/or H_2S, Urease production, Citrate utilization test, Motility, Indole production, Ornithine decarboxylation and esculine hydrolysis. Identification of the strains was confirmed using API 20E/ID32E (BioMérieux)

Detection of some virulence factors of isolated Proteus spp

The ten isolated *Proteus* strains were subjected to the following tests; three different types of motility; swarming (SW), swimming (SM) and twitching(TM), urease production, biofilm formation and the ability to swarm over three different types of urinary catheters (latex, silicon, silicon coated latex).

Motility tests

Luria–Bertani (LB) medium (Sigma-Aldrich, USA) with different agar contents was used for detection of different types of motility. Swimming medium contained 0.3% agar. Swarming medium contained 0.7% agar. Twitching medium contained 1.0% agar. After cultivation for 24 hrs at 37 °C, the diameters of produced zones will be measured (Rashid and Kornberg, 2000, Jones et al., 2004).

Urease production test

Proteus isolates were inoculated using a pick on plates of Christensen medium (Oxoid, UK) and cultivated for 24 hrs at 37°C aerobically. The diameter of the formed pink zone was assessed (Stankowska et al., 2008).

Swarming over three different types of urinary catheters

LB swarming Medium was used for this test. From the agar plate, a strip of the medium was aseptically cutaway. The prepared gap was bridged with 2cm pieces of the tested sterile catheters: "latex Foley catheter (20 Fr, Well Lead Medical company, China), all silicon Foley catheter (20 Fr, Hospital and Homecare Medical Device company, China), silicone-elastomer coated latex Foley catheter (20 Fr, Well Lead Medical company, China)".

The *Proteus* isolates were inoculated using a sterile cotton swab on one-half of the agar. After 24 hrs of cultivation at 37 °C aerobically, the ability to swarm over the tested catheter was assessed. The strains were divided into three groups: (0) non-swarming strains, (1) swarming strains not able to bridge the catheter, and (2) strains able to bridge the catheter (Jones et al., 2004, Sabbuba et al., 2003).

Biofilm formation test

Latex catheters (22 F) were aseptically cut into approximately 2-cm pieces and each piece was submerged in 2 mL brain heart infusion (BHI) (Oxoid, UK) in sterile tubes with 4% glucose (BHI-g) and others without glucose, and then each tube was inoculated with 200 µL suspensions of the tested *Proteus* strains. After 24 hrs cultivation at 37 °C, the catheters were aseptically removed from the medium, washed three times with sterile phosphate-buffered saline (PBS) (PH=7.2) (Sigma-Aldrich, USA) to wash out non-adherent cells and transferred into fresh BHI. The biofilm of adherent cells disrupted by sonication and vortex technique (5 min sonication, 2 min vortexing, 5 min further sonication) (Compe`re et al.,

2009). The used ultrasonic bath for sonication was (BRANSON 3510, USA) and Barnstead/Thermolyne Maxi-Mix, test tube mixer (VWR, USA) was used for vortexing. The significant level of biofilm formation is ≥1000 CFU/ml for the vortexing and sonication techniques, according to the quantitative technique of (Brun-Buisson et al., 1987). A 0.1-ml of each cultivated BHI tubes with catheter pieces was added to either 0.9 ml(1:10 dilution) and 9.9 ml (1:100 dilution) of saline and vortexed. Then, 1 µL of these dilutions and 1 µL of the sonicated broth were surface plated by using calibrated loops on Xylose lysine deoxycholate (XLD) agar (Oxoid, UK).

All plates were incubated aerobically for 24 hrs at 37°C, and then the number of CFU were counted. Accurate and significant colony counts are 10^3, $>10^3$-10^6,10^7 for undiluted BHI, 1:10 dilution and 1:100 dilution, respectively. The previous counts indicate that this strain was biofilm former in different dilutions while counts below this range were classified as non-biofilm former (Sherertz et al., 1990). The following international reference strains were used as controls for biofilm formation: *Staphylococcus aureus* ATCC6538P (very strongly biofilm forming strain) and *E. coli* ATCC 35218 (weakly biofilm forming strain). (liofilchem)

Antibiotic susceptibility testing of isolated Proteus

Disc diffusion methodon Muller-Hinton agar (Oxoid, UK) was used according to (CLSI, 2015). The used antibiotics discs (Oxoid, UK) were amikacin (10µg), gentamicin (30µg), trimethoprim / sulfamethoxazole (1.25/23.75 µg), nalidixic acid (30µg), cephalothin (30 µg), cefazolin (30µg), cefotaxime (30µg), norfloxacin (10µg), ciprofloxacin (5µg), imipenem (10µg), ampicillin (10µg) and amoxicillin / clavulanic acid (20/10µg).

Statistical Analysis

Data were analyzed using the software, Microsoft Excel 2007and IBM SPSS software package version 20.0. Qualitative data were described using numbers and percentage. Quantitative data were described using Range (minimum and maximum), mean, standard deviation and median. Comparisons between different groups regarding categorical variables were tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's exact test or Monte Carlo correction. P-value for Student t-test compared between the studied groups was detected with statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The most important adverse outcome of using indwelling urinary catheter is CAUTI. Our study showed that 72.7% of patients with indwelling urethral catheter developed CAUTIs and this agreed with other studies (Okafor et al., 2005, Hazelett et al., 2006, Kang et al., 2015) whose resultswere72%, 74% and 73.4%, respectively. This high level may be due to increase unnecessary usage of indwelling urinary catheters and improper maintenance of them. (Table 1)

In the current study, the mean ± SD of age of CAUTI cases was 59.5±14.5 years. This was inconsistent with a study that found the mean± SD of age was 56.0 ± 18.3 years (Tambyah and Maki, 2000) and Markovic-Denic et al., (2011) found that the mean age was 64.9 years. This result may be due to that (53/80, 53.75%) of CAUTI cases were isolated from adult ICUs and (27/80, 33.75%) of them from urology outpatient clinics especially who had benign prostatic hyperplasia (BPH). (Table 1)

As regard risk factors, CAUTI was detected more in men than women without statistical significance (p-value = 0.967) and this is supported by Knežević et al., (2010) which detected that the most CAUTI (79.3%) was in elderly male patients and Philips, (2014) found no relation between sex and CAUTI while other study found that CAUTIs in women(66%) more than in men (34%) with $p < 0.001$(Tambyah and Maki, 2000). (Table 1)

In this research, it was found that the long duration of catheterization is an important risk factor as the mean ± SD of duration of catheterization of CAUTI cases was 15.08±8.6 days that was statistically significant difference in comparison with non-CAUTI cases ($p < 0.001$) and this agreed with another study whose result was 32.93 ±36.23 days (Temiz et al., 2012) and also, Barbadoro et al., (2015)found that the duration of catheterization>4 days was an important risk factor for CAUTI. Also, Hooton et al., (2010) revealed that the duration of catheterization is the most important determinant of-bacteriuria. (Table 1)

Another risk factor for developing CAUTI was D.M, we found that diabetic patients were (26/80 cases, 32.5%) of CAUTI cases that was statistically significant in comparison with cases of non-CAUTI cases ($p=0.006$), this result agreed with another study that found diabetic patients were 15% of positive CAUTI cases (Temiz et al., 2012), so diabetic patients are more susceptible to biofilm formation and stone formation as a sequence than non-diabetic ones**.** (Table 1)

In our study, it was found that (66/80 cases, 82.5%) of CAUTI cases were taken antibiotics especially third generation cephalosporin. Another study revealed that60–80% of hospitalized patients with indwelling catheters used antimicrobials, usually for other indications (Hooton et al., 2010). (Table 1)

In the present study, the most common isolated uropathogens from CAUTI were *Candida spp* (29/80 isolates, 36.25%) (Table 2) and this was in accordance with the study done by Temiz et al., (2012) who found that the most common isolated pathogens were *Candida spp* (33.3%) and (Chang et al., 2011) found high candidal infection in their study (19.7%)**,** also. This result may be due to increased empirical use of antibiotics by patients included in the study (66/80 cases, 82.5%) and (26/80 cases, 32.5%) of them were diabetic. Other studies agreed with that *E.coli* is the most isolated organism from CAUTIs (Ortega et al., 2013, Sader et al., 2014).

In this research, *Proteus* isolates were accounted for (10/80 cases, 12.5%) of CAUTIs (Table 2) and this agreed with others who found *Proteus spp* in 14%, 11.47% of patients with CAUTIs, respectively (Hung et al., 2007, Essomba et al., 2013).

In this study, 60% of isolated *Proteus* strains were *P.mirabilis* and 40% were *P. vulgaris* but *P.–penneri* was not detected from CAUTIs. This agreed with the study that found *P.mirabilis* was responsible for 65% CAUTIs isolates while *P. vulgaris* was responsible for 31.7 % and this result came close to our results (Kim et al., 2003).

While testing some virulence factors of those ten *Proteus* isolates, we found that all of them were urease producer, had the ability to swarm and swim all over the plates except one strain didn't swarm all over the plate (Figure 1) and this is in agreement with these studies (Mu¨ller, 1986, Hola et al., 2012). Also, all of them were able to twitch between two solid surfaces and this disagreed with the study done by Hola et al., (2012) that showed that 33.3% of their isolated *Proteus* from CAUTI formed a twitching zone of 2–3 cm. Our result may indicate that *Proteus* isolates in our study were more virulent.

Table 1. Demographic data of CAUTIs and non-CAUTIs cases

Demographic data	CAUTIs N =80 (72.7%)	Non CAUTIs N=30 (27.2%)	Total (n=110)	P value
	N (%)	N (%)		
Departments				
• **Urology**	27(33.75)	4(13.33)	31(28.1)	0.034*
• **Adult ICUs**	53(66.25)	20(66.66)	73(66.3)	0.967
• **Pediatric ICU**	0(0)	6(7.5)	6(5.4)	p <0.001*
Sex				
• **Male**	46(57.5)	12(40)	58(52.7)	0.102
• **Female**	34(42.5)	18(60)	52(47.2)	
Age (y)				
• **Min. –Max.**	19-95	1-86	1-95	p <0.001*
• **Mean± SD**	59.5±14.5	43.3±27.5	55.11±20.2	
• **Median**	60	50	60	
Duration of catheterization(d)				
• **Min. –Max.**	3 – 45	3-24	3 - 45	p <0.001*
• **Mean ± SD**	15.08±8.6	8.4 ±4.5	13.26 ±8.3	
• **Median**	12.5	8	10	
Antibiotic intake				
• **Yes**	66(82.5)	22(73.33)	88(80)	0.284
• **No**	14(17.5)	8(26.66)	22(20)	
DM				
• **Yes**	26(32.5)	2(6.6)	28(25.45)	0.006*
• **No**	54(67.2)	28(93.3)	82(74.5)	

CAUTI: catheter-associated urinary tract infection**, ICU**: intensive care unit, **y**: years, **d**: days, **DM**: diabetes mellitus, statistically significant at $p \leq 0.05$

Table 2. Types of isolated organisms from CAUTIs cases

Isolated uropathogenic organisms	CAUTI (n=80)	
	N	%
Candida spp	29	36.25
Klebsiella spp	19	23.75
Eischerichia coli (E.coli)	18	22.5
Pseudomonas aeuroginosa	12	15
Proteus spp	10	12.5
Staphylococcus aureus	3	3.75
Diphteroid spp	1	1.25
Serratia marcescens	1	1.25
Acinetobacter baumannii	1	1.25

CAUTI: catheter-associated urinary tract infection
P. mirabilis were six and *P. vulgaris* were four of *Proteus* isolates while *P. penneri* was not detected among the isolates.

Table 3. Swarming over three different types of catheters

Isolated *Proteus* strains (N=10)	Types of the catheter		
	Latex Foley catheter	Silicon Foley catheter	Silicon coated latex Foley catheter
Non swarming strains (N) %	(0) 0	(0)0	(0)0
swarming strains not able to bridge the catheter (N) %	(6)60	(8)80	(8)80
swarming strains able to bridge the catheter (N) %	(4)40	(2)20	(2)20
p		0.952(0.628)	0.952(0.628)

Statistically insignificant at p ≥0.05

Table 4. Antibiotic sensitivity of isolated Proteus strains from CAUTIs by disc diffusion method

Antibiotics (µg)	S (mm) (N)%	I (mm) (N)%	R (mm) (N)%
Amikacin(30)	(6)60	(0)0	(4)40
Gentamycin (10)	(5)50	(0)0	(5)50
Trimethoprim/sulfamethoxazole (25)	(0)0	(2)20	(8)80
Nalidixic acid (30)	(3)30	(0)0	(7)70
Norfloxacine (10)	(4)40	(4)40	(2)20
Ciprofloxacin(5)	(5)50	(3)30	(2)20
Cephalothine (30)	(0)0	(0)0	(10)100
Cefazoline(30)	(0)0	(0)0	(10)100
Cefotax(30)	(3)30	(2)20	(5)50
Imipenem (10)	(5)50	(3)30	(2)20
Amoxacillin /clavulinic acid (20/10)	(0)0	(2)20	(8)80

S: sensitive, **I:** intermediate, **R:** resistant

While testing the ability of isolated *Proteus* to swarm over three different types of urinary catheters, it was found only two cases were able to bridge silicon Foley catheter and–silicone-elastomer coated latex Foley catheter while four cases could bridge over latex Foley catheter (Table 3, Figure 2). The difference was statistically insignificant (p=0.628) and this may be due to the low number of tested *Proteus* isolates in our study. This result indicated that catheter made of silicon is better than latex ones. These results were in accordance with Jones et al., (2004) that tested migration of *P. mirabilis* over the surfaces of urinary catheters, their experiments showed that migration occurred more readily over hydrogel-coated latex sections than over all-silicone sections. About biofilm formation, by using the quantitative sonication technique, according to Burn-Buisson et al., (1987), we found that all *Proteus* isolates could form biofilm in undiluted BHI. This agreed with Kwiecinska-Piróg et al., (2014) who found that biofilm formation was detected by all the examined *P. mirabilis* strains which isolated from urine.

Our study revealed that biofilm formation was enhanced by using BHI with 4% glucose more than BHI without glucose (p = 0.036) (Figure 3) and this agreed with results of other studies that found biofilm formation of *Proteus i*solates from CAUTI was increased with glucose supplementation (Hola et al., 2012). Other studies showed that supplementation of the medium with glucose increases the ability of *Staphylococci* to form biofilm (Baldassarri et al., 2001, Mathur et al., 2006). These results indicate that diabetic patients show a high risk for developing biofilm and blockage of the indwelling urethral catheter due to high glucose level in their urine.

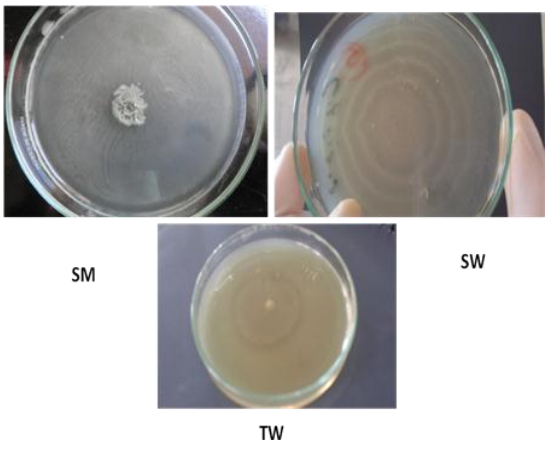

Figure 1. *Proteus*isolates shows swimming(SM) on LB 0.3% agar, swarming (SW) on LB 0.7% agar, twitching (TW) on LB 1.0% agar contents.

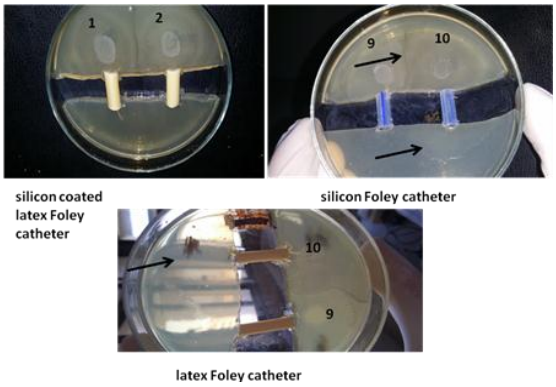

Figure 2. Swarming over three different types of catheters (latex, silicon and silicone-coated latex Foley catheters). Dienes line is formed between two different *Proteus* isolates, some isolates could bridge and others couldn't.

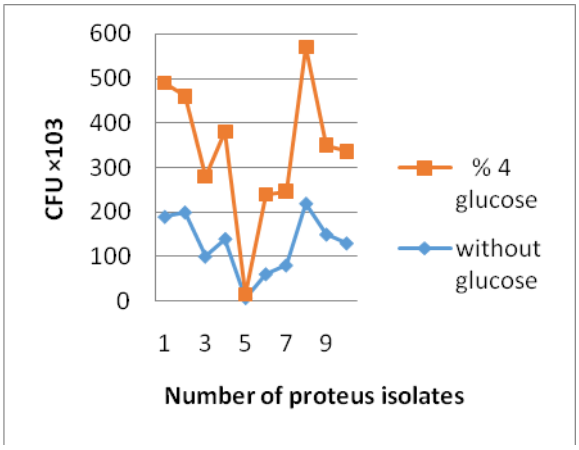

Figure 3. Correlation between biofilm formation using BHI with and without 4% glucose.

As regard pattern of antibiotics resistance of *Proteus* isolates from CAUTI to different classes of antibiotics; the highest resistance was to cephalothine and cefazoline (100%) while susceptibility to amikacin, gentamicin and ciprofloxacin as the following 60%, 50%, 50%, respectively (Table4). This result disagreed with a recent study done by Wang et al., (2014) that revealed the following rates of susceptibility to the same antibiotics 90.5%, 85.7%, 68.7%, respectively. The differences in antibiotic sensitivity pattern might be due to the differences in usage of antibiotics in different countries and the misuse of antibiotics in our country due to cultural and economic factors.

In this study, MDR was found in seven cases and XDR was detected in two cases of *Proteus* isolates from CAUTI while only one strain was sensitive. The appearance of this percentage of MDR and XDR is a major problem and we have to follow wise antibiotic policy to overcome those resistant organisms. This was in accordance with Philips, (2014) who found that spread of MDR organisms are representing a growing public health problem in the world, so there is a need for regular review of antimicrobial pattern among *Proteus* isolates for accurate decision on antibiotic prescription.

CONCLUSIONS

The results of this study showed that it is necessary for prevention of CAUTIs by limit using urinary catheter and if inserted, remove it as early as possible and if you suspects long usage of urinary catheter, it's preferred to use silicon catheters instead of latex ones. We can conclude that the risk factors for CAUTI are male, old age, long duration of catheterization, D.M and previous antibiotic intake. *Proteus* isolates from CAUTI were very virulent and most of them were MDR, so studying more virulence factors of this genus helps us to prevent or limit infections caused by them.

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