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ORIGINAL ARTICLE

Bacterial Profile in Urine, Burns, and Wounds in Diabetic and non-Diabetic Patients

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ABSTRACT

Background: *The risks that threaten diabetic patients as a result of bacterial infection exceed than those in normal persons. This is clearly seen in skin, urinary tract and wound infections, which require special medical care and increase the cost of treatment, especially when these infections are accompanied by wide resistance to many antibiotics and the possession of resistance genes that can be transmitted to other bacteria.* ***Objectives:*** *Are to determine the prevalence of antibiotics resistant bacteria in diabetes.* ***Methodology:*** *One hundred and thirty bacteria were isolated from urine, burns and wounds from diabetes and non-diabetes in two hospitals in Alexandria, Egypt. The were screened for their antibiotics resistance. These isolates were tentatively identified related to Staphylococcus aureus and named M22 "diabetic skin", Pseudomonas aeruginosa named M76 "diabetic skin" and E. coli which named M113 "diabetic urine". These three isolates were selected for further investigations, since they achieved the highest resistance to traditional antibiotics. Isolate M22 was resistant to Methicillin, isolate M76 and M113 showed more resistance to Ampicillin. PCR was carried out for detection of Mec A gene in S. aureus, and β-lactamase gene in Pseudomonas aeruginosa and E. coli.* ***Results:*** *The presence of Methicillin resistant gene (MRSA) have confirmed by PCR as well as the ampicillin resistant genes in Pseudomonas aeruginosa and E. coli. The resistant genes were confirmed by the presence of distinguished bands at 858 bp and 850 bp respectively.* ***Conclusion:*** *Special cares should be considered and taken to prevent or reduce bacterial infections of diabetics.*

INTRODUCTION

Diabetes mellitus increases patient's susceptibility to various infections. The most common sites of infection in diabetic patients are the skin and urinary tract. Malignant or necrotizing otitis externa principally occurs in diabetic patients older than 35 years and is almost due to *Pseudomonas aeruginosa* [1]. Diabetic foot ulcers is a common complication of diabetes and frequently associated with the presence of *Staphylococcus aureus*[2]. Most UTIs (urinary tract infections) caused by bacteria are thought to occur more frequently in diabetes. The emphysematous infections, which sometimes occur, refer to those complicated by gas formation due to bacterial fermentation [3].

The affected areas of the infected Skin and soft tissues may become dysfunctional (eg, hands and legs) according to the infection severity. In some cases, the mild infections may rapidly convert into life threat infection as a result of diabetes mellitus and AIDS [4].

The skin in the principal barrier against microbial invasion is colonized with a diverse of microbial populations. The vast majority of these colonized flora are the bacteria. The typical organisms that colonize the skin above the waist are usually Gram-positive species such as *Staphylococcus epidermidis, Corynebacterium species, S. aureus* and *Streptococcus pyogenes*[5]. The latter two species are particularly significant because they contribute to a majority of SSTIs. Chronic wound infection is a major clinical problem that leads to high morbidity, mortality and cost. It has been reported that no distinction is seen between the diabetic and non-diabetic patients in burn cases in the visual issue, since both had corneal scraped spots, glaucoma, cataracts, or blindness. [6]

Prompt diagnostics and susceptibility testing, early and aggressive surgical and/or antibiotic therapy are important factors for treatment of antibiotic-resistant infections in diabetic patients [7]. Sustained infections were developed when diabetic persons were wounded than those non-diabetics, and the chance of invasive co-infections with endogenous bacteria was increased [8].

Methicillin –Resistant *Staphylococcus aureus* (MRSA) which were described firstly in the 1960s. During the late 1970s and early 1980s, strains of *S. aureus* resistant to multiple antibiotics including methicillin and gentamycin were increasingly responsible for outbreaks of hospital infections worldwide and several clonal types have shown extensive international spread [9, 10].

β-Lactam resistant *Pseudomonas aeruginosa,* the worldwide emergence of multi-drug resistant bacterial strains in hospitals and community continues to be a problem of due scientific concern, especially infections caused by *Pseudomonas* species and *Pseudomonas aeruginosa* in particular. *P. aeruginosa* is an opportunistic pathogen with inherent resistance to many antibiotics and disinfectants including anti-pseudomonal Penicillin, Ceftazidime, Carbapenems, Aminoglycosides and Ciprofloxacin [11, 12].

Extended-spectrum β-lactamase (ESBL)–producing *E. coli* increased worldwide, but still uncommon in USA. *E. coli* is considered the most common β-Lactam resistant bacteria that infect the urinary tract (80%–90%). [13]

The aim of the present work is to identify the bacterial profile in some infections of the diabetic and non-diabetic patients.

METHODOLOGY

Sampling

Five hundred and thirty-five (535) samples were collected from diabetic and non-diabetic patients attending Mabaret El Asafra (311 samples) and Alexandria General Hospitals (224), Egypt for 12 months (2011) as shown in table 1. Samples were obtained from the hospital laboratories, which were taken under aseptic conditions; urine was taken in sterile cups and samples from burns and wounds were taken by sterile swabs, and then transferred to laboratory. All procedures were carried out in accordance with ethical guidelines by Ethics Committee.

Table 1: Numbers of clinical specimens collected from two hospitals in Alexandria - Egypt

Wounds		Burns		Urine		Total	
Diabetic	Non-diabetic	Diabetic	Non-diabetic	Diabetic	Non-diabetic	Diabetic	Non-diabetic
6	23	66	71	147	222	219	316
29		**137**		**369**		**535**	

Bacterial isolates

From urine, Burn and Wounds

Bacteria were isolated from 369 urine samples by plating 1 ml of urine on three nutrient agar (NA)[14]. Bacteria were isolated from 166 wounds and burns samples as well[15].

Identification of bacterial Isolates

A hundred and thirty (130) Isolates were identified tentatively[16]. The identification was confirmed using API 20 NE, API20E and Protein A Latex. Isolates then named; M22, M76 and M113 and selected for further investigation, these isolates were identified as *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli* respectively.

Antibiotics susceptibility test

The assay was performed by using disc diffusion methods and the inhibition zone was measured [17].

Genomic DNA Extraction

Genomic DNA of *Staphylococcus aureus* (M22), *Pseudomonas aeruginosa* (M76) and *E. coli* (M113) was extracted by using phenol-chloroform method and separated electrophoretically on 1.5% agarose and visualized by UV-trans-eliminator [18].

Detection of Methicillin resistance gene

Methicillin resistance gene in *Staphylococcus areus* was detected (Mec A gene)[19], and Beta-lactame resistant gene of *Pseudomonas aeruginosa*as as well[20,21]. Ampicillin resistant gene of *E. coli* as was also detected by using PCR techniques [22].

RESULTS

Isolation of Bacteria

As shown in table 2, 130 isolates were isolated from 535 samples; 80 isolates from diabetic wounds, urine and burns, and 50 isolates from non-diabetics.

No *E. coli* was isolated from burns or wounds, but only from urine. *Staphylococcus aureus* was the most isolated species from wounds and burns in diabetic (38.75%) and non-diabetic (58%) as well. Most of identified bacteria were isolated from diabetic (61.5%).

Table 2: Numbers and origins of bacterial isolates

Hospital / Strains	Mabbart El Asafra Hospital			Alexandria General Hospital			Total Isolates	Diabetes	Non Diabetes
	Burns	**wounds**	**Urine**	**Burns**	**Wounds**	**Urine**			
Staphylococcus aureus	30	10	-	20	-	-	60	31	29
Pseudomonas aeruginosa	10	-	20	10	-	10	50	26	24
E.coli	-	-	10	-	-	10	20	9	11
Total isolates	**40**	**10**	**30**	**30**	**-**	**20**	**130**	**80**	**50**

Antibiotic Susceptibility Test

As shown in table 3, *Staphylococcus aureus* (M22) was resistant to 87.5% of the antibiotics used in this study, whereas *Pseudomonas aeruginosa* (M76) was resistant to 43.75%, and *E. coli* (M113) was resistant to 50%.

Table 3: Antibiotics susceptibility for tested isolates M22, M76 and M113

Tested Isolate / Tested Antibiotics	Antibiotics Concentration	M22 *Staphylococcus aureus*	M76 *Pseudomonas aeruginosa*	M 113 *E. coli*
Amoxicillin	10 µg/ml	+++	+++	+++
Ampicillin	10 µg/ml	+++	+++	+++
Carbenicillin	10 µg/ml	++	-	-
Chloramphenicol	30 µg/ml	++	-	-
Ciprofloxacin	5 µg/ml	-	-	-
Clindamycin	10 µg/ml	++	-	-
Erythromycin	15 µg/ml	++	-	-
Gentamycin	10 µg/ml	++	+++	+++
Methicillin	5 µg/ml	+++	+++	+++
Oxacillin	10 µg/ml	+++	++	+++
Penicillin G	10 unit	+++	-	++
Streptomycin	10 µg/ml	-	-	-
Tetracycline	30 µg/ml	++	-	-
Tobramycin	10 µg/ml	++	+++	+++
Vancomycin	10 µg/ml	++	++	++
Oxytetracycline	30 µg/ml	++	-	-

(-) sensitive (++) intermediate resistance (+++) Resistant.

As shown in table 4, the most effective antibiotics towards the tested strains were oxitetracycline 99.22%, Streptomycin 97%, chloramphenicol 97.7%, ciprofloxacin 97.7%, clindamycin 96.2%, and penicillin G 92%. The ratio refers to the sensitive isolates.

Table 4: The susceptibility pattern of antibiotics of 130 isolates isolated from urine, burns and wounds

Tested Antibiotics	No. of isolates	Resistant (%)	No. of isolates	Sensitive (%)
Amoxicillin	48	37.00	82	63.00
Ampicillin	49	37.70	81	62.30
Carbenicillin	19	14.60	111	85.40
Chloramphenicol	3	2.30	127	97.70
Ciprofloxacin	3	2.30	127	97.70
Clindamycin	5	3.78	125	96.20
Erythromycin	33	20.40	97	79.60
Gentamycin	44	33.80	86	66.20
Methicillin	44	33.80	86	66.20
Oxacillin	38	29.20	92	70.80
Penicillin G	17	8.00	113	92.00
Streptomycin	4	3.00	126	97.00
Tetracycline	15	11.60%	115	88.40
Tobramycin	44	33.80	86	66.20
Vancomycin	27	20.70	103	79.30
Oxitetracycline	1	0.78	129	99.22

Resistant isolates include resistant and intermediate resistant

Figure 1, shows the resistance pattern in diabetes (66%) was higher than those in non-diabetes (34.

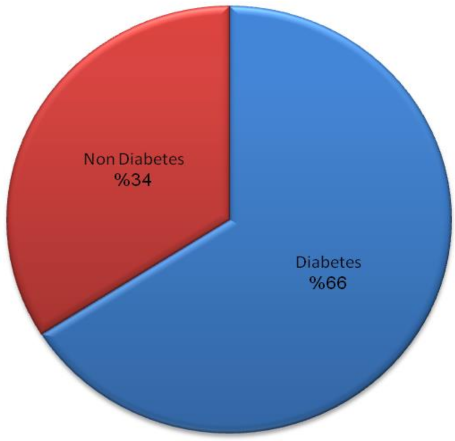

Fig. 1: Resistance pattern in diabetes and non-diabetes

Methicillin Resistance gene of *Staphylococcus aureus* M22

A bands of 162 Kb, that distinguished to *Mec A* gene were visualized in the bacteria used (Fig. 2)

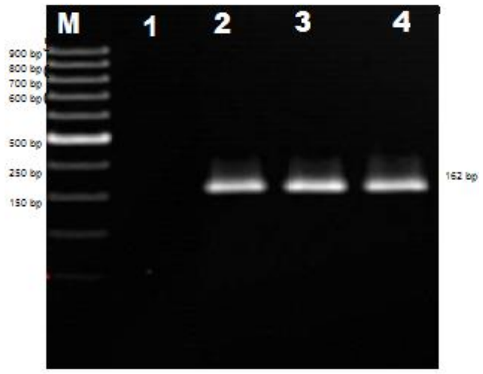

Fig. 2: Methicillin resistance genes. lane M: DNA ladder marker, 1: negative MRSA strain, 2: positive control previously identified as MRSA strain, 3: Strain M22 and 4: isolate no. 47 MRSA positive.

Ampicillin Resistance gene of *Pseudomonas aeruginosa* M76

β-lactamase resistance genes blaTEM and blaSHV were investigated, however, the genes were detected in the *Pseudomonas aeruginosa* that appeared to be 858 bp in size (Fig. 3).

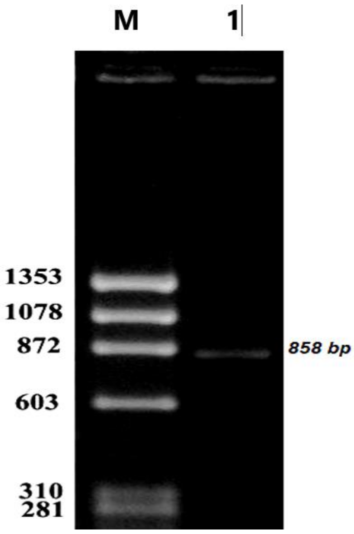

Fig. 3: β- Lactam Resistance gene of *Pseudomonas aeruginosa* M76.
M: DNA ladder. 1: Strain M76.

Ampicillin Resistance gene of *E. coli* M113

850 bp band was detected as an amplified Ampicillin resistant gene using polymerase chain reaction (Fig. 4).

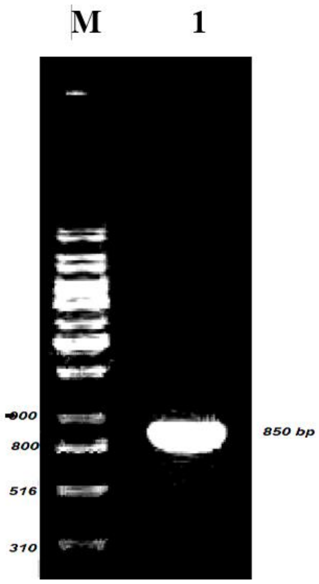

Fig. 4: Ampicillin resistance gene of *E. coli* M113 lane 1

DISCUSSION

In our study, isolates that were identified as *Pseudomonas aeruginosa*, were the most common organism encountered in urine that represent 60% of the isolates. In a similar study, *Pseudomonas aeruginosa* was responsible for 10.7% of infections of 141 pathogens from hospital-acquired infections [23]. In 2014, 30% of the patients (both diabetics and non-diabetics) presented with asymptomatic bacteriuria and the prevalence of pyelonephritis were significantly higher (p=0.04) in diabetics compared to non-diabetic patients[24].

In Malaysia, it was concluded that *Pseudomonas aeruginosa* (19%), *Staphylococcus aureus* (11%) and bacteroides species (8%) were isolated from diabetic foot infections[1].

In a similar study, the most frequently isolated bacteria were *S. aureus*, which represent 51.56% in 33 samples[25]. It has been concluded that Gram-positive aerobic bacteria were the most common micro-organism (56.7%) isolated form wounds followed by Gram-negative aerobic bacteria and anaerobes (29.8% and 13.5%, respectively)[26]. *S. aureus* was the most common organism found, and 40% were MRSA, which agreed with the results of the present study.

Tentolouris *et al.*[27], they also reported similar results, 36% of isolates were resistant to more than one group of antibiotics. They reported that 75% of the isolates were susceptible to ciprofloxacin, 73% to amikacin; 65% to ceftazidime; 63% to meropenem; 63% to imipenem; 60% to piperacillin/tazobactam; 59% to cefoperazone/sulbactam; 54% to cefepime and 44% to tobramycin. The majority of carbapenem resistant isolates were susceptible to ciprofloxacin and amikacin as well, which corresponded with our results in the current study.

Reports documented that the highest number of *Pseudomonas* infections was found in urine, followed by pus and sputum and the maximum sensitivity of the organism was against the carbapenems [28]. These results also agreed with our study in the same area.

Our results corresponded with those concluded later in 2014 [29], since the prevalence of metallo-β-lactamases (MBL) and extended-spectrum β-lactamases (ESBL) in *P. aeruginosa* isolates.

Using PCR to identify MRSA is more effective, when 439 swabs, using combination of MRSA, tested for presence of *mecA* gene encoded the extra penicillin binding protein[30]. Use of a broth-PCR method for detection of MRSA had been described [31] previously and had been implemented for routine screening for MRSA colonization, and these results agreed with the present results.

CONCLUSION

Epidemiological studies should use PCR-based detection tests followed by analysis of the PCR products by sequencing or restriction with endonucleases chosen to detect restriction site changes generated by point mutations.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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