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## ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA FROM DIFFERENT CLINICAL SAMPLES COLLECTED FROM EGYPT, SAUDI ARABIA, AND SUDAN

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### ABSTRACT

Pathogenic bacteria contribute to many globally important diseases, such as pneumonia, urinary tract infection, foodborne illnesses, and others. The present study aims to study and survey different isolates of pathogenic bacteria from different countries to assess their prevalence and their relation to diseases. Two hundred and ninety-two clinical samples were collected randomly from private laboratories and Hospitals of three countries (Egypt, Saudi Arabia, and Sudan). All samples were collected from patients (male and female) for ages from one to 89 years during the period 2015-2016. Samples included the present study were urine (168), vaginal swab (43), ear swab (22), blood (19), abscess (17), endotracheal tube (8), sputum (8), throat swab (3), nasal swab (3), and urethral swab (1). All samples were inoculated on different selective and differential culture media. After growth, isolated bacteria were identified by physiological and biochemical properties. Mainly 5 bacterial genera were detected amongst all clinical samples. The isolates were identified to be *Escherichia coli* (103), *Klebsiella* spp. (47), *Proteus* spp. (14), *Pseudomonas* spp. (83), and *Staphylococcus* spp. (45). Regarding patient gender, the presented study showed that females had a tendency to get infected more than males, where 110 (37.67 %) of patients were males and 182 (62.33 %) were females. The most common microbes in urinary tract infection were *Escherichia coli*. The most common microbes in acute otitis externa were *Pseudomonas aeruginosa* and *Staphylococcus aureus*. All isolates from abscesses were *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The results showed that *Pseudomonas aeruginosa* was the most common isolated samples and only *Proteus mirabilis* were isolated urine samples. Survey and studies for infectious agents are one of the most significant epidemiological tools to track infectious disease and to foretell disease patterns, as well as determining the distribution of agents according to body parts. Pathogenic bacteria contribute in many globally important diseases.

**Key words:** Isolation; bacterial identification; Pathogenic bacteria; clinical samples.

### 1. INTRODUCTION

Due to the frequent and easy travel of people between countries, especially by flying, the chance of spreading pathogenic bacteria is easy [1]. As a result of early transport networks and population expansion, many pathogens of some places have moved everywhere [2]. The origins of emerging infectious diseases are largely linked to economic, social and environmental factors. About 54.3% of emerging infectious diseases are caused by bacteria or rickettsia [3]. Emerging infectious diseases increase over time in number and abundance of causal diseases, despite infectious

diseases in general, global action on prevention, early detection and treatment is more effective in reducing the number of infected people [4].

Globally, *E. coli* is the most common pathogen causing urinary tract infection, it causes 80–85% of cases [5, 6]. It also causes other diseases such as Foodborne illnesses [7], and pneumonia [8]. It is sometimes found in abscesses and skin windows, especially in the lower body [9].

*Klebsiella pneumoniae* is the most relevant human pathogen within genus *Klebsiella* spp., causing many infections worldwide, including lung, abdominal cavity, urinary tract, soft tissue

infections, and surgical sites, even bacteremia [10]. It is the species of aerobic Gram-negative bacteria most commonly renowned as a cause of community-acquired pneumonia [11]. It is the most important microorganism causes urinary tract infection, next to *Escherichia coli* [10].

*Pseudomonas aeruginosa* is an opportunist microbe and it is one of urinary tract infection microbes [12]. It is even the third most common pathogen-related with hospital-acquired catheter-associated urinary tract infections [13]. It can sometimes cause community-acquired pneumonia [14, 15, 47]. *Pseudomonas aeruginosa* is considered one of the most important organisms that cause otitis media [16], cutaneous infection by it range from superficial to deep [17].

*Proteus mirabilis* is most frequently pathogen of the urinary tract, especially in patients undergoing long-term catheterization [18]. The most common clinical appearance of *Proteus* infection is urinary tract infections and ninety percent of *Proteus* infections occur as a result of *Proteus mirabilis* [19].

*Staphylococcus aureus* is significantly isolated from urine samples obtained from long-term care patients [20]; it can cause pneumonia [21]. It is most frequently found in chronic suppurative otitis media [22, 48], and most commonly identified agent responsible for skin and soft tissue infections worldwide, causing furuncles and skin abscesses [23]. Consequently, it is assumed that there will be a continuous follow-up to reveal the extent of these pathogens and see whether they are increasing or not by comparing recent results with the results of previous studies. Therefore, this study aimed to isolate, identify and characterize of bacterial pathogens from Egypt and surrounding countries Sudan and Saudi Arabia to assess their prevalence and their relation to diseases.

## 2. MATERIAL AND METHOD

### 2.1. Samples collection

Two hundred and ninety-two samples were collected randomly from three countries Egypt, Saudi Arabia, and Sudan, distributed as the following 90, 162 and 40, respectively. The

specimens were collected from private laboratories and hospitals from different genders and ages from one to 89 years in the period between 4/2015–7/2016.

### 2.2. Cultivation of samples

The samples were transferred safely into the lab according to microbial guide transfer [31]. The samples were inoculated on different media by streaking method; then incubated overnight at 37°C. The cultured media for bacterial isolates were Cysteine–lactose–electrolyte-deficient agar (C.L.E.D) its composition Gms / Litre: Peptic digest of animal tissue 4.0, Casein enzymic hydrolysate 4.0, Beef extract 3.0, Lactose 10.0, L-Cystine 0.128, Bromo thymol blue 0.02, and agar 15.0 [24, 50]; Sheep blood agar, its composition Gms / Litre: Tryptone 14.0, Peptone 4.5, Yeast extract 4.5, Sodium chloride 5.0, Defibrinated sheep blood 70.0 and agar 12.5 [25]; MacConkey agar its composition Gms / Litre: Peptone 20.0, Lactose 10.0, Bile salts 5.0, Sodium chloride 5.0, Neutral red 0.075 and agar 12.0 [26], Chocolate agar its composition Gms / Litre : Peptone 20.0, Dextrose 0.5, Sodium chloride 5.0, Disodium phosphate 5.0, Sterile Lysed blood (at 80°C) 50.0 and agar 15.0 [27], and Thayer martin agar, its composition Gms / Litre: Peptic digest of animal tissue 23.0, Starch 1.0, Sodium chloride 5.0, Dextrose 2.5 and agar 20.0 [28].

### 2.3. Isolation and identification

Bacteria have been isolated from pure colonies then examined microscopically by using Grams stain technique to distinguish between Gram-negative and Gram-positive bacteria [29]. The identification tests including the cultural, morphological, and physiological properties of each bacterial isolate were done [30].

Biochemical tests used for bacterial isolates including the following: Catalase, Coagulase, Novobiocine sensitivity, Oxidase, Indole, Citrate and Urease tests, Glucose, Lactose and Mannitol fermentation, also gas and H<sub>2</sub>S production [31].

### 2.4. Statistical analysis

All data included were analyzed statistically using the statistical package, IBM SPSS version 23.

### 3. RESULTS

The presented study including samples was collected from urine, blood, vaginal swab, ear swab, abscess, nasal swab, endotracheal tube, urethral swab, throat swab, and sputum.

#### 3.1. Distribution of samples according to gender

A total of 292 samples have been collected to be investigated, 182 (62.3%) were females and 110 (37.7%) were males (Fig.1). Regarding countries distribution, in Egyptian samples, 37.8% were females and 62.2% were males. For Saudi Arabia, 81.5% of total samples were females and 18.5% were males. Whereas for Sudan samples, 40% were females and 60% were males (Fig. 2).

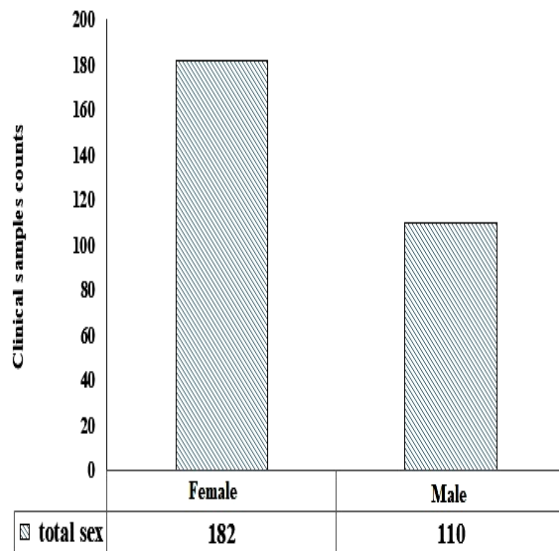


Fig. (1) Total distribution of samples according to gender.

#### 3.2. Samples distribution according to specimen's types

Out of total specimen's types, the majority of the abscess, blood, ear swab, nasal swab, and throat swab specimens were obtained from males as the following (94.1%), (57.9%), (59.1%), (66.7%) and (66.7%), respectively. While the most urine specimens obtained from females which were (68.5%). Regards to endotracheal tube samples were divided equally between males and females. In the presented study, the sputum and urethral swab samples were obtained only from males (Fig. 3).

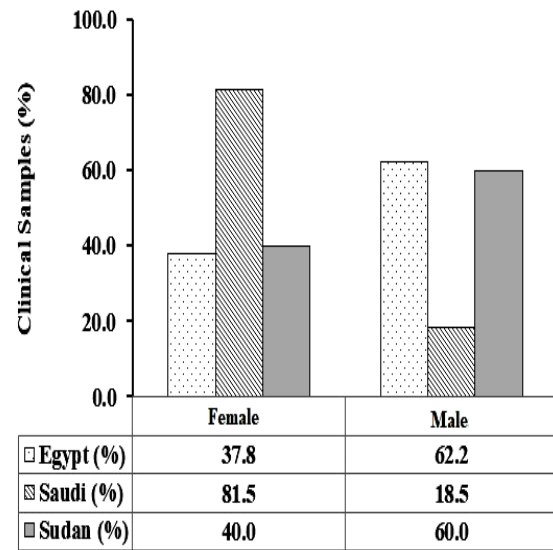


Fig. (2) Percentage of clinical samples distribution in Egypt, Saudi Arabia and Sudan according to gender.

#### 3.3. Distribution of isolates according to samples type

Urine culture samples represented the majority of samples collected with 168 (57.5%), followed by vaginal swab 43 (14.7%), ear swab 22 (7.5%), blood 19 (6.5%), abscess 17 (5.8%), endotracheal tube 8 (2.7%), sputum 8 (2%), throat swab 3 (1%), nasal swab 3 (1%), and urethral swab 1 (0.34%) (Fig.4).

Urine culture samples collected from Egypt were the major with (47.8%), followed by blood (21.1%), abscess (10%), endotracheal tube (8.9%), sputum (8.9%), nasal swab (2.2%), throat swab (1.1%), and urethral swab (0.6%), while there is no vaginal swab and ear swab.

For Saudi specimens, majority of the bacteria were isolated from urine culture with (56.8%), followed by vaginal swab (25.9%), ear swab (11.1%), abscess (3.7%), throat swab (1.2%), and nasal swab (0.6%), while blood, endotracheal tube, sputum, and urethral swab were not present.

Regards to Sudan, the majority of the bacteria were isolated from urine culture with (82.5%), followed by ear swab (10%), abscess (5%), and vaginal swab (2.5%), while blood, endotracheal tube, sputum, throat swab, nasal swab, and urethral swab samples were not present (Fig. 5).

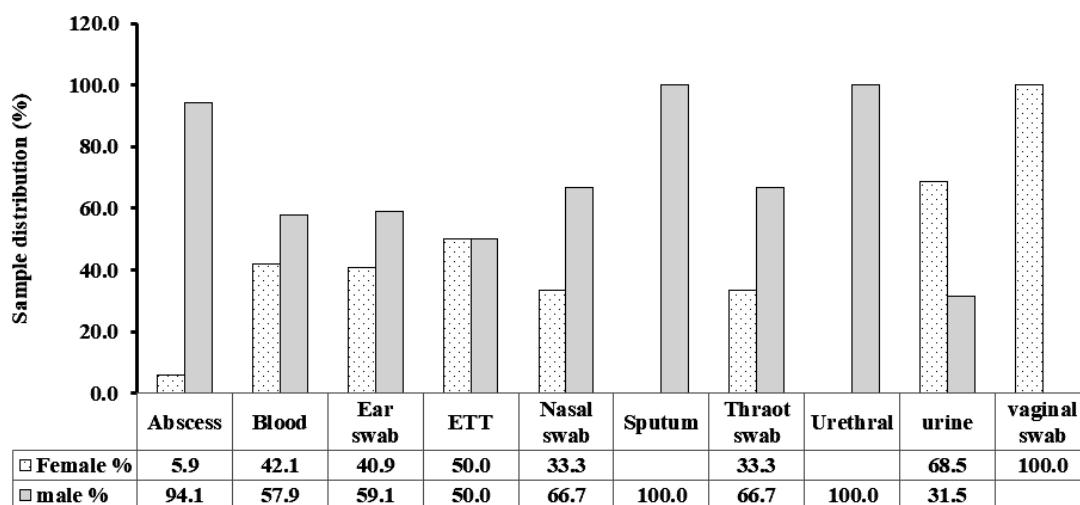


Fig. 3: percentage distribution of clinical samples according to gender.

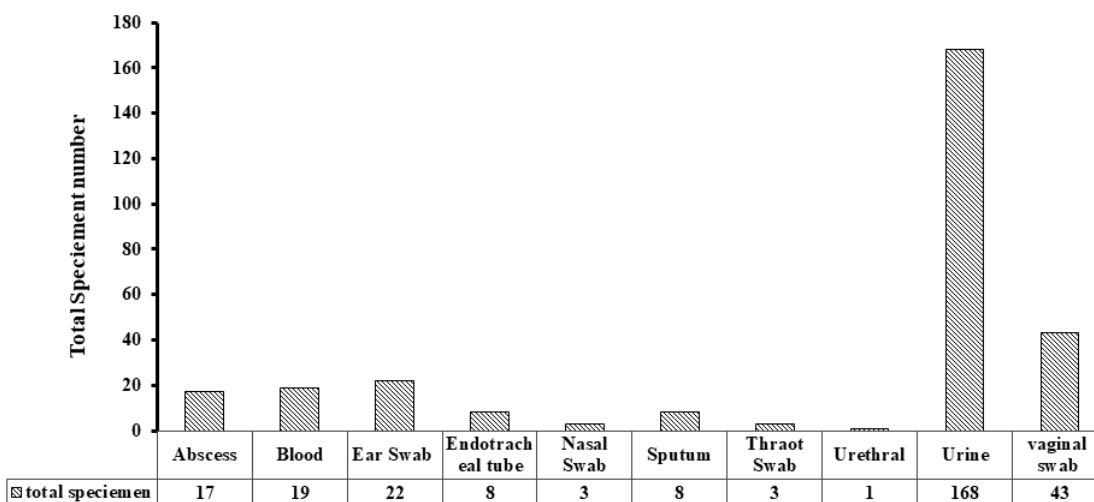


Fig. 4: Distribution isolates of clinical samples according to origin of isolation.

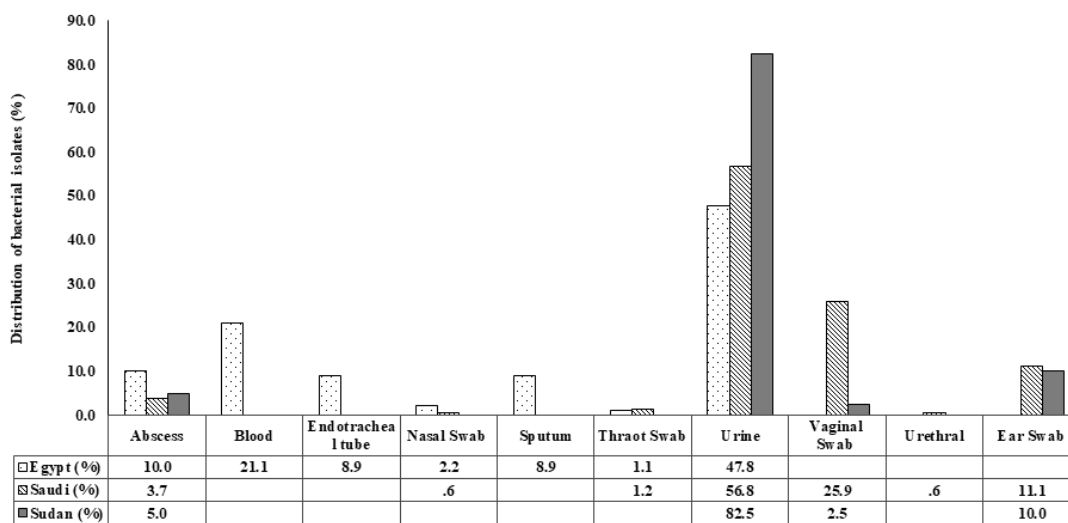


Fig. 5: Geographic distribution of clinical isolates according to samples type

### 3.4. Identification of isolates

From the different clinical samples identified bacteria, *E. coli* were predominant with 103 (35.3%), followed by *Pseudomonas* spp. 83 (28.4%), *Klebsiella* spp. 47 (16.1%), *Staphylococcus* spp. 45 (15.4%), and *Proteus* spp. 14 (4.8%) (Fig.6). The majority of isolated bacteria from Egypt were *E. coli* (36.7%), followed by *Pseudomonas* spp. (26.7%), and *Staphylococcus* spp. (18.9%), and *Klebsiella* spp. (17.8%), while there is no *Proteus* spp. For Saudi Arabia, the most common were *E. coli* (33.3%), followed by *Pseudomonas* spp. (25.9%), *Staphylococcus* spp. (17.3%), *Klebsiella* spp. (14.8%), and *Proteus* spp. (8.6%). The most common isolates obtained from Sudan were *Pseudomonas* spp. (42.5%), followed by *E. coli* (40%), and *Klebsiella* spp. (17.5%), while *Proteus* spp. and *Staphylococcus* spp. were not detected (Table 1).

### 3.5. Distribution of isolated bacteria among samples

Out of total isolated bacteria, *Pseudomonas* spp. were the most isolates obtained from abscess and window swab (64.77%), followed by *Staphylococcus* spp. (29.4%) and *E. coli* (5.9%), while there is no *Proteus* spp. and *Klebsiella* spp., while the majority of isolates obtained from blood were *Staphylococcus* spp. (57.9%) followed by *Klebsiella* spp. (31.6%), *E. coli* (5.3%), and *Pseudomonas* spp. (5.3%), while there is no *Proteus* spp. Out of ear swab, the present isolated bacteria were *Pseudomonas* spp. (54.5%) and *Staphylococcus* spp. (45.5%), while there is no *Proteus* spp., *E. coli* or *Klebsiella* spp. Among isolates obtained from the endotracheal tube, *Pseudomonas* spp. (62.5%) were the most, followed by *E. coli* (12.5%), *Klebsiella* spp. (12.5%), and *Staphylococcus* spp. (12.5%), while there are no *Proteus* spp. Isolated bacteria from nasal swab

Table (1) Distribution and percentage of the obtained bacterial isolates in Egypt, Saudi Arabia and Sudan.

Country	Egypt		Saudi Arabia		Sudan	
	No. of isolates	%	No. of isolates	%	No. of isolates	%
<i>E. coli</i>	33	36.7	54	33.3	16	40.0
<i>Klebsiella</i> spp.	16	17.8	24	14.8	7	17.5
<i>Proteus</i> spp.	0	0	14	8.6	0	0
<i>Pseudomonas</i> spp.	24	26.7	42	25.9	17	42.5
<i>Staphylococcus</i> spp.	17	18.9	28	17.3	0	0

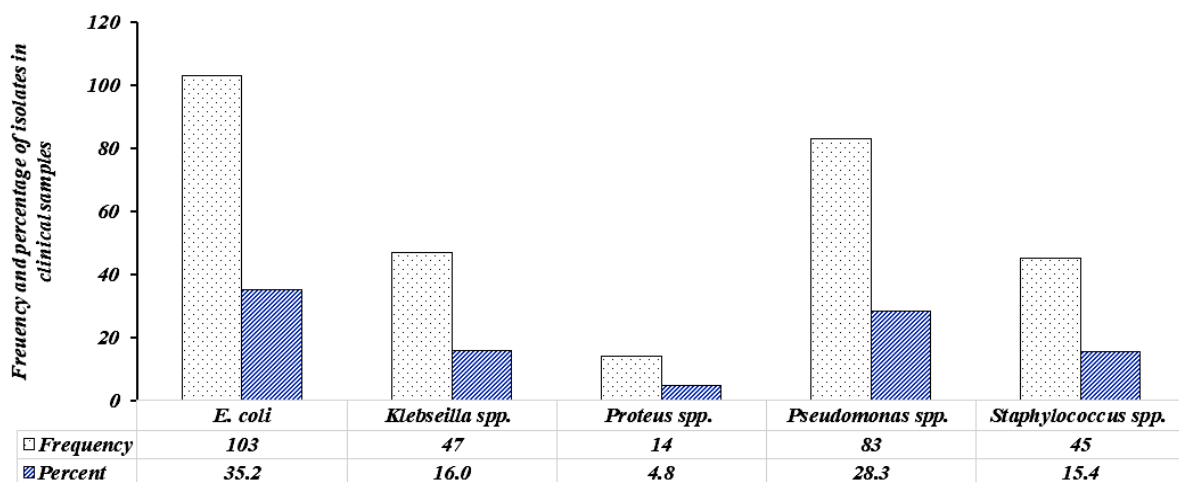


Fig 6: Frequency and percentages of bacterial isolates in clinical samples.

were *Pseudomonas* spp. (33.3%) and *Staphylococcus* spp. (66.7%), while there is no *Proteus* spp., *E. coli* or *Klebsiella* spp. Out of sputum specimens, the majority isolates were *Klebsiella* spp. (50%), followed by *Pseudomonas* spp. (37.5%), and *E. coli* (12.5%), while there is no *Proteus* spp. or *Staphylococcus* spp. Isolated bacteria from throat swab were *Klebsiella* spp. (33.5%) and *Pseudomonas* spp. (66.7%) only, while *Proteus* spp., *Staphylococcus* spp. and *E. coli* were not isolated. Only one isolate was obtained from urethral swab that is *Staphylococcus* spp. (100%). The largest number of isolates from urine were *E. coli* (55.4%), followed by *Klebsiella* spp. (19%), *Pseudomonas* spp. (15.5%), *Proteus* (8.3%) and *Staphylococcus* spp. (1.8%). Finally, vaginal swab isolates were *Pseudomonas* spp. (51.1%) as the most, followed by *Staphylococcus* spp. (27.9%), *E. coli* (14%), *Klebsiella* spp. (7%), and there is no *Proteus* spp. (Fig. 7).

#### 4. DISCUSSION

Pathogen bacteria cause many infectious diseases and spread worldwide such as respiratory tract infections, urinary tract infections, and others. In general, women are more susceptible to infectious diseases than men for several reasons: biological differences as well as social differences and cultural norms [32]. In the present study, results show that women are the most infected cases of microbial disease, however, upon detailing; we reported

that the result in Saudi Arabia is consistent with the overall result, but in Egypt and Sudan is different as men are the most infected cases. In our study total results indicate that males are more infected by respiratory infections than females. This corresponds to the available data which indicate that males are more infected with respiratory infections than females, especially the lower respiratory system, otitis media, and croup, the role of sex hormones in the organization of the immune system may also participate to the reported sex differences in the incidence and gravity of the various types of respiratory tract infections, especially in adolescents and adults [33]. In this study, *Pseudomonas* spp. was isolated from all specimens' types except urethral swab, and they are the second most isolates after *E. coli*. In other studies, *Pseudomonas* spp. as an opportunistic microbe attacks people who have weakened immunity, affecting the respiratory system, urinary system, gastrointestinal system, wounds, eye, bone, burns, and blood [34]. The reason is that *P. aeruginosa* possesses an overabundance of virulence factors that facilitate the capability of this pathogen to cause a various array of infections in humans [35].

We have noticed from our study that *Proteus* spp. isolated only from urine samples. Other studies reported that the most common clinical appearance of *Proteus* infection is urinary tract infections and ninety percent of *Proteus* infections occur as a result of *Proteus*

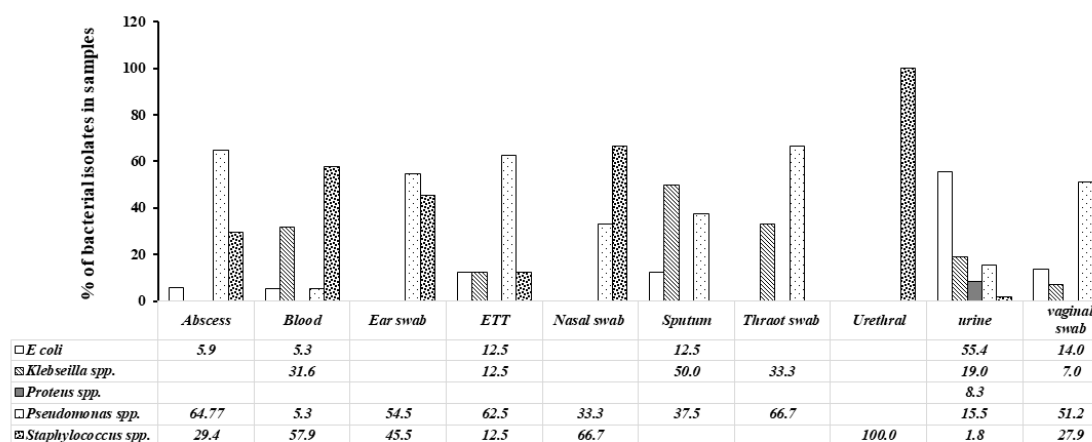


Fig. (7) Distribution of isolates bacteria according to samples types

*mirabilis* [19]. Also in our study, the lowest percent of isolates were *Proteus* and were isolated only from Saudi samples. Other studies refer to that in healthy human, *Proteus* accounts for 1% to 2% of all urinary tract infections, while in hospital-acquired UTIs, *Proteus* accounts for 5% [19], because the Saudi samples are the most rations among the samples, they were expected to appear in them only.

In the presented study the most common isolates in urine were *Escherichia coli*, followed by *Klebsiella* spp. Studies indicated that the gastrointestinal Gram-negative flora is the well-determined causes of most urinary tract infections globally [37], and the most common microbes in urinary tract infection are *Escherichia coli*, and *Klebsiella* spp.[5, 37]. Urinary tract infection in females is also more than doubled if compared to males. According to studies women are more prone to urinary tract infection than men, due to their anatomy; the urethra in females is much smaller and earlier to the anus [38].

In the presented study, all isolates from the ear were *Pseudomonas* spp. and *Staphylococcus* spp. Some studies said that acute otitis external is caused primarily by a bacterial infection, *Pseudomonas* spp. and *Staphylococcus* spp. are the most common [39]. Previous studies have explained that *P. aeruginosa* and *S. aureus* invades human middle ear epithelial cells [40, 41]. Two strains were isolated from abscesses are *Staphylococcus* spp. and *Pseudomonas* spp. The studies refer to abscesses are caused by some responses but the bacterial infection is the most common cause [42]. Although, there are often several microbes in one abscess, the majority is *Staphylococcus* spp. [43, 44, 49]. *S. aureus* produces several molecules that contribute to the formation of abscesses [45]. Among the Gram-negative organisms, *P. aeruginosa* represented the second most common pathogen in nosocomial susceptibility in skin infections [46]. *P. aeruginosa* has an increase of features that contribute to its ability to cause disease, several proteases facilitate *P. aeruginosa* dissemination through allowing the microorganism to disrupt basement membrane

safety, also phospholipase C activity and other factors contribute to virulence [17].

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### 6. Conflict of interest statement

Authors declare that there are no conflicts of interest.

### 7. Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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## تحديد وتوزيع البكتيريا الممرضة في العينات الطبية داخل ثلاث دول مجاورة : مصر والمملكة العربية السعودية والسودان

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### الملخص العربي

يهدف هذا البحث إلى مسح ودراسة وتعريف وتوصيف عزلات مختلفة من البكتيريا المسببة للأمراض تم عزلها من مناطق جغرافية مختلفة لتقييم مدى انتشارها وعلاقتها بالأمراض.

تم جمع مائتين واثنين وتسعين عينة بشكل عشوائي من عدة مختبرات ومستشفيات خاصة في ثلاث دول متجاورة (مصر والسعودية والسودان). تم جمع العينات من مرضى من الجنسين من فئات عمرية مختلفة خلال الفترة 2015-2016.

كانت عينات هذه الدراسة عبارة عن (168) عينة بول ، (43) مسحة مهبلية ، (22) مسحة أذن ، (19) عينة دم ، (17) عينة خُرَاج ، (8) عينات أنبوب القصبة الهوائية ، (8) عينات بلغم ، (3) مسحات حلق ، (3) مسحات أنف ، ومسحة واحدة من مجرى البول الذكري. تم زراعة جميع العينات على وسائط غذائية مناسبة لكل عينة . تم وضع العينات المزروعة في درجة حرارة 37 درجة مئوية لمدة 24 ساعة ، بعد عملية النمو تم تحديد وتعريف البكتيريا المعزولة بواسطة معرفة الخصائص الفسيولوجية ونتائج الإختبارات البيوكيميائية لكل عينة.

أسفرت نتائج التعريف عن وجود خمس أجناس بكتيرية بشكل رئيسي بين جميع العينات السريرية وكانت هذه الأجناس عبارة عن أربعة أجناس سالبة الجرام وهي : 103 عزلة من الإيشريشيا كولاى (*Escherichia coli*) ، 47 عزلة من سلالات الكليبيسيلا (*Klebsiella spp.*) ، 14 عزلة من سلالات بروتياس (*Proteus spp.*) ، 83 عزلة من سلالات السيدوموناس (*Pseudomonas spp.*) ، وجنس واحد فقط من البكتيريا موجبة الجرام وكانت 45 عزلة من المكورات العنقودية الذهبية (*Staphylococcus spp.*). كما أظهرت نتائج الدراسة المُقدَّمة أن الإناث لديهم ميل للإصابة أكثر من الذكور ، حيث كانت العينات عبارة عن 110 (37.67%) من الذكور في حين كان 182 (62.33%) من الإناث. كذلك كانت أكثر أنواع الميكروبات شيوعًا في عدوى المسالك البولية هي الإيشريشيا كولاى. وكانت أكثر الميكروبات شيوعًا في التهاب الأذن الوسطى الحاد هي سلالات السيدوموناس والمكورات العنقودية الذهبية. وكانت جميع العزلات من الخراجات هي السيدوموناس والمكورات العنقودية الذهبية. كما أظهرت النتائج أيضًا أن السيدوموناس كانت أكثر العينات المعزولة شيوعًا ، وكانت جميع عزلات البروتياس من عينات البول فقط.