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Urinary tract infection and non-ruptured acute appendicitis association: Uro-pathogens findings

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Abstract. Urinary Tract Infections (UTIs) consider as the most common infections worldwide, with higher risk in patients experienced Acute Appendicitis (AA). The purpose of this study was, to investigate the bacterial profile of UTIs in patients with non-ruptured AA post-surgically, and to assess age- and gender-related links of all AA cases in Karak region, Jordan. Urine samples obtained from 46 cases (32 male and 14 female) aged between 16-70 years were diagnosed as non-ruptured AA, following with isolation and characterization of isolated bacteria. Out of 46 AA cases, uropathogens isolated from 25 (54.3%) UTI cases. Out of these isolates; 42 (73.7%) were gram-negative isolates and 15 (26.3%) were gram-positive bacteria. The percentage of isolates were *E. coli* (26.3%), *Enterobacter* species (21%), *Enterococcus faecalis* and *Klebsiella pneumoniea* (10.5%) for each, *Streptococcus saprophytics* and *Pseudomonas aeruginosa* (7%) for each, *Yersinia* spp. and *S. milleri* (8.8%). Out of UTI cases, 20 cases (80%) possessed mixed culture, each of them had at least one of Enterobacterial species. *i.e. Enterobacter* spp. or *E. coli* or both. More precisely, out of all these positive-cases, 2 cases had pure gram positive-bacterial infection (8%), while pure gram negative bacterial infection comprised 48% of them and the rest (44%) were mixed (gram-negative and gram-positive) bacterial infection. Moreover, study revealed a high prevalence rate of AA cases 24 (52.2%) in the ages of 16-22 years, then declining the rate with increasing the age, reaching the lowest rate (4.3%) in ages of 60-70. In addition to age factor, the males significantly more susceptible to AA cases than females by 2.2-fold. Antibiotic sensitivity test revealed high resistance capability of *E. coli* to the most used antibiotics except for nitrofurantoin. Bacterial isolates showing sensitivity against ciprofloxacin, trimethoprim/sulfamethoxazole, amoxicillin-Clavulanic acid and nitrofurantoin, with a superiority for the first two. Results demonstrate high prevalence rate of UTIs in patients with AA. For avoiding, the needless use of antibiotics through sticking to our accountability as healthcare provisioner to pursuit the antimicrobial management.

INTRODUCTION

Acute appendicitis (AA) is one of the most common abdominal emergency affects people in different ages, with more incidence rate between the ages 10 and 20 years (Petroianu, 2012). Signs of AA ranged between tenderness to sharp pain in the right lower abdominal quadrant (Schick *et al.*, 2011). Obstruction of appendix with fecal matter or lymphoid hyperplasia lead to bacterial overgrowth and so pus-filled appendix; acute appendicitis (Rothrock and Pagane, 2000; Schick *et al.*, 2011). As progression of the AA inflammatory fluid and bacterial contents might escape to the blood stream and peritoneal cavity, consequently spreading of pathogens to the secondary sites (Gilchrist *et al.*, 2011; Mackay *et al.*, 1987). Studies revealed that AA is a disease of all ages with higher tendency for younger patients (aged $\leq$ 29 years) (Harbrecht *et al.*, 2011).

Moreover, it was revealed that AA with a superiority for males rather than females, with a ratio from 1.4:1 to 3:1 (Petroianu, 2012).

Different pathogens have been reported in AA, including viral, fungal, and parasitic infections (Lamps, 2008). Among these pathogens, bacterial species have implicated as the major causative agent in AA, including both aerobic and anaerobic, gram-negative and gram-positive bacteria (Gilbert *et al.*, 2002). Bacterial species including; *Streptococci* spp. (Jacobsen *et al.*, 1987), *Yersinia enterocolitica* (Okoro, 1988), *E. coli* (Guasco *et al.*, 1991), *Klebsiella* spp. (Leigh *et al.*, 1974), *Citrobacter frundii* (Parkhomenko *et al.*, 1991), and *Bacteroids fragilis* (Gilbert *et al.*, 2002). Not only these aforementioned bacterial species were identified, but also other species have been isolated later from AA; including *Pseudomonas*, *Salmonella*, *Shigella*, *Actinomycis*, *Campylobacter*, *Mycobacterium*, *Rickettsia*, *Streptococcus* (Chan *et al.*, 2010; Lamps, 2008).

Due to close anatomical proximity of appendix to the urogenital tract, pathogens of AA might spread to the retroperitoneal space, and so may invade the urinary tracts (Puskar *et al.*, 1997). Therefore, some bacterial species were isolated from urinary culture in patients with appendicitis such as: *E. coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Enterobacter*, *Klebsiella*, *Enterococcus faecalis* and *Corynebacterium* species (Arnbjörnsson, 1988; Gardikis *et al.*, 2002; Puskar *et al.*, 1997). Most previous studies have focused on the bacterial profile of appendicitis, while few studies have assessed bacteriology of urinary tract system in patients with AA. Therefore, this study was designed to deduce if there is a bacteriological association between appendicitis and both urinary tract infection (UTI) and blood infection. In addition, during present research, gender and age factors and their association with the prevalence of AA has been demonstrated.

Design and Methodology

The subjects and sample collection

Urine and blood samples were collected post-operatively from each case of the 46 persons (32 male and 14 female; ages between 16-70 years) admitted to Al-Karak Governmental Hospital and Prince Ali Ebn-Elhassan Hospital and have confirmly diagnosed as non-ruptured AA. Blood samples, were taken by vein puncture technique, placed in sterile bottles and transferred immediately in icebox to the laboratory for bacterial isolation. While, urine samples were transported immediately to laboratory within less than half hour in sterile urine collection containers. In fact, all the cases where admitted to the hospital and underwent emergency surgery (appendectomy) and have not received antibiotic therapy previously.

Additionally, differentiation between the asymptomatic bacteriuria and true UTI was made via history of the symptoms and preformed a mid-stream sample of urine (MSU). Asymptomatic bacteriuria who show no symptoms of urinary tract: Female patients with Urine depicted bacterial growth $\geq 10^5$ cfu/mL in two successive samples, and male patients with Urine depicted bacterial growth $\geq 10^5$ cfu/mL in a single sample. Meanwhile, Patients with bacterial growth as low as $(10)^2$ cfu/mL in urine with symptoms of urinary tract, such as urgency, frequency, dysuria, pyuria, haematuria, and nocturia, were considered to exemplify true bacteriuria (UTI) in both gender (Nicolle *et al.*, 2005 and Warren *et al.*, 1982).

Inclusion and exclusion criteria

In all the reported cases admitted to the hospital, confirmed diagnosis of perforated AA were based on laboratory findings, radiological modalities and postoperative histological findings (Körner *et al.*, 2001), in which the doctor final decision for each case include was non-perforated AA. Patients aged 16 -70 years, with confirmed diagnosis as non-perforated AA; has been included during the study. Some criteria had been excluded including; pregnant, diffused appendicitis. Moreover, for eligible study patients with previous commenced antibiotic treatment for UTI have been excluded.

Bacterial isolation

A suspension was made via adding 1 ml from collected sample to 9 ml normal saline

containing test tube. The suspension was serially diluted (1:10) with sterile saline solution up to 10^{-4}. 100 µl from the decimal dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were cultured on nutrient agar, blood agar and MacConkey agar media and incubated over night at 37°C. Isolation of bacterial strains was carried out based on morphological and blood hemolysis characteristics. Only the cases with significant bacteriuria (>10^5 cfu/ml) were considered for the microbiological analysis. Bacterial isolates were stored at 4ºC, and sub-cultured monthly for long term perseveration.

Characterization and Identification of bacterial strain

Isolation and identification of bacteria

Upon arrival to the laboratory, the samples were cultured on the nutrient agar. The pure colony that resulted from the first inoculation was cultured into four plates of MacConkey agar, mannitol salt agar, eosin methylene blue and blood agar to select and differentiate the resulted colony. These entire five agars were incubated at 37°C and checked after 24 and 48 h to workout CFU/mL. Urine cultures were incubated overnight aerobically at 37°C (Vandepitte *et al.*, 2003). Bacterial identification was made using standard Gram stain procedure, cultural characteristics such as colony morphology and biochemical tests (Holt *et al.*, 1994). To obtain the biochemical characteristics for these isolates, different identification kit systems have been used. These identification kit systems consist of (1); Microgen GN -ID system, which consists of 12 substrates only and which are selected specifically to improve the most common identification of negative Bacilli including the Enterobacteria and Acinetobacter spp. A combination of Microgen GN A + GN B identification systems was used to determine the common intestinal syndrome from urinary specimens that were Gram-positive oxidase positive. Bacterial culture was examined by spotting and oxidase testing prior to use of the GN-ID Microgen system. (2), the *Bacillus*-ID Microgen system developed to determine *Bacillus* spp. and related species. Bacterial culture was examined by the oxidase test before using the *Bacillus*-ID Microgen system. (3), Microgen Strep-ID is a biochemical testing system that uses 12 microwell test (12 test) and 3 out-of- strip tests; hippurate (provided), alpha-hemolysis and beta-hemolysis for the identification of Streptococcal and Enterococcus species. (4), Microgen Staph-ID for Staphylococcus spp. Gram positive, catalase positive, and latex/coagulation testing were performed as pre-tests. The substrates used specifically for Staphylococcus aureus and related species were selected for results in 24 hours.

Antimicrobial susceptibility testing (Kirby-Bauer method)

All cultivated isolates were tested for their susceptibility by using eight specific antibiotics using standard method, as set by the National Committee for Clinical Laboratory standards, 2005. Susceptibility test was performed by using those 8 commonly used antibiotics, including with their concentration in µg/disc: (trimethoprim/sulfamethoxazole (TRI-SU, 1.25/23.75 µg), Cefixime (CFX, 5), Amoxicillin-clavulanic acid (AM-CL, 20/10), Ampicillin (AMP, 10), ciprofloxacin (CIP, 5), gentamycin (GEN, 10), nalidixic acid (NAL, 30) NIF, nitrofurantoin (NIF,50). All antibiotic discs were purchased from SPAN Diagnostic Ltd, Udhna, India. Bacterial isolates were inoculated in peptone water and later incubated in 37°C. After one day, by swabbing technique new cultures were platted on Mueller-Hinton agar. Then, plates were incubated for 10-15 min at 37°C. Finally, by using a sterile forceps, the investigated disks were placed on the cultured plates and incubated for 24 h at 37°C (Qaralleh *et al.*, 2009).

Statistical analysis

Harvesting data and statistical One-way ANOVA using Graph Pad Prism 7.05 (Graph Pad Software, Inc., La Jolla, CA, USA). In which $p < 0.05$ was considered as significant result analyzed values.

RESULT AND DISCUSSION

Isolation of bacterial strain from blood sample

Out of 46 AA persons, four cases showed positive cultures (2 females, 2 males) demonstrated bacterial growth on blood agar; *Bacteriodes fragilis* and *Klebsiella pneumoniae*. These findings were in accordance with many previous studies, for example, Roberts (Roberts, 1988), reported that *B. fragilis* has a role as a secondarily invasive bacteria causing appendicealsepsis. Moreover, it was documented that septicemia with *Klebsiella pneumoniae* may be attributed to the bacterial translocation through appendix mucosa leading in dissemination of the infection into blood stream (Salemis, 2009). Although, there are no correlation between AA and blood sepsis (Lau *et al*., 1984), it was reported that low incidence of blood infection aids the theory of retroperitoneal spreading of colonic bacteria from appendix to adjacent organs, such as urogenital tracts (Puskar *et al*., 1997).

Bacterial isolates from urine samples

Twenty-five (54.3%) out of 46 urine samples revealed positive bacterial growth on the agar plates. From these samples, 57 isolates were obtained according to morphological and biochemical characteristics. Our results are in agreement with Yamamoto *et al*. (Yamamoto *et al*., 1985); they demonstrated that 47-53% of appendicitis cases have abnormal urinalysis due to ruptured or abnormally positioned appendix. Out of all isolates; 15 (26.3%) were gram-positive bacteria and 42 (73.7 %) were gram-negative isolates. Based on Gram staining, biochemical characterization and microbiological identification kits, the bacterial isolates were assigned to 8 different bacterial species (Tables 1 and 2).

Study revealed that gram-negative bacteria were the prevailing isolates (73.6%) over the gram-positive ones in the urine samples. In the same manner, most previous studies suggested that the common pathogens of UTI are mostly prevailed by gram-negative bacteria with high percentage that could reach to 75-90% (Hooton, 2012; Kline and Lewis, 2016). Moreover, bacterial profile of AA demonstrated that gram-negative bacteria are major pathogens, due to high capability of gram-negative bacteria for adhesiveness and colonization as compared with that of gram-positive bacteria (Naher and Ktab, 2013). In overall, the uropathogens resulted from gram negative bacteria are prevailing with high rate of *E. coli* (26.3%) followed by Enterobacter species (21%). These results were in parallel with many previous investigations suggested the superiority of *E.coli* and other enteric bacteria that generate UTIs (Al-Asoufi *et al*., 2017; Khleifat *et al*., 2006). In addition,

Table 1. Bacterial isolates from 46 AA cases, fifty-seven bacterial isolates were yielded from 25 urine samples. Isolates was identified as gram positive 15(26.3%) and gram-positive bacteria 42(73.7%)

Bacterial isolates	(n=57)	Mean total bacterial count (cfu/ml)
Gram positive bacteria	**15 (26.3)**	
Staphylococcus saprophyticus	4 (7)	47 x10^2
Enterococcus faecalis	6 (10.5)	35 x10^2
Streptococcus milleri	5 (8.8)	32 x10^2
Gram negative bacteria	**42 (73.7)**	
Escherichia coli	15 (26.3)	40 x10^3
Yersinia sp.	5 (8.8)	20 x10^1
Enterobacter sp.	12 (21)	58 x10^3
Klebsiella pneumoniae	6 (10.5)	26 x10^1
Pseudomonas aeruginosa	4 (7)	32 x10^2

Table 2. Isolated bacteria (N=57) in urine samples for each case. *(+, gram positive bacteria / -, gram negative bacteria)

Case No.	Gram-negative bacteria					Gram-positive bacteria			Number of Isolates*/Case
	Pseudomonas aeroginosa	*Klebsiella pneumoniae*	*Enterobacter* sp.	*Yersinia* sp.	*E. coli*	*Streptococcus milleri*	*Enterococcus faecalis*	*Staphylococcus saprophyticus*	
1		■			■	■		■	2- / 2+
2	■		■		■				3-
3			■						2-
4	■				■	■			2- / 1+
5		■	■	■				■	3- / 1+
6					■		■		1- / 1+
7			■						1-
8		■			■		■		2- / 1+
9			■						1-
10	■			■	■				3-
11							■		1+
12			■		■				2-
13		■			■		■	■	2- / 1+
14				■	■	■			2- / 1+
15			■						1- / 1+
16				■	■				2-
17		■			■		■		2- / 1+
18	■		■			■			-2 / +1
19					■			■	1+ 1-
20			■						1-
21			■		■				2-
22							■		1+
23			■		■				2-
24			■	■		■			2- / 1+
25		■			■				2-

findings were in agreement with the bacterial profiles of appendix, in which that most frequently isolated bacteria were enteric gram-negative bacilli including *E. coli*, *Enterobacter* and *Klebsiella* (Bennion *et al.*, 1990; Lahiri, 2016). This may because *E. coli* and *Enterobacter* account the commonest flora in the intestine, which capable for fast proliferation and adhere to the tissue surface (Baron *et al.*, 1994). Therefore, the prevailing infection by these bacteria is due to their capability to spread from appendix to the retroperitoneal spaces invading urinary tract (Puskar *et al.*, 1997). There is many another virulence factors enable *E. coli* for being in a position of major distrusting like toxins, haste-cell-surface-modifying factor, cyto-toxin necrotizing factor and hemolysin (Beach, 1981). All these etiological findings suggest the superiority of gram-negative bacteria in both AA and UTI, with high correlation rate between the behavior of colonic flora (origin of gram-negative bacteria) and the incidence of both diseases. In addition, many other uropathogens were recorded including 10.5% for *Enterococcus*

Table 3. Antibiotic sensitivity profiles for all cultivated isolates

Antibiotic	*S. saprophyticus*	*E. faecalis*	*S. milleri*	*E. coli*	*Yersinia* sp.	*Entero.* sp.	*K. pneumoniae*	*P. aeroginosa*
TRI-SU	–	–	+/–	+/–	–	+/–	–	–
CFX	+/–	+	+	+/–	+/–	–	+/–	+/–
AM-CL	–	–	–	+/–	–	+/–	–	–
AMP	–	+	–	+	+	–	+	+
CIP	–	+/–	–	+/–	–	–	–	–
GEN	–	–	+/–	+	–	+/–	–	+/–
NAL	+	+/–	+	+/–	+	+/–	–	+/–
NIF	+/–	–	–	–	+/–	–	+/–	–

SXT, trimethoprim/sulfamethoxazole; CFX, Cefixime; AM-CL, Amoxicillin-Clavulanic acid; AMP, ampicillin; CIP, ciprofloxacin; GEN, gentamycin; NAL, nalidixic acid; NIF, nitrofurantoin. +, Resistant; –, Sensitive; +/–, Partially Sensitive.

faecalis and *K. pneumoniea*, 7% for *S. saprophytics* and *P. aeurogonosa* and 8.8% for *Yersinia* spp. and *S. Milleri*, these results were in agreement with that of previous studies (Arnbjörnsson, 1988; Lahiri, 2016; Mohammed *et al.*, 2017).

As result, among all positively urine-cultured cases (n=25), mixed cultures were detected in 20 cases (80%), in which more than one isolate has been detected; 1-4 for each case (Table 2). Although, some studies considered these mixed culture as contaminated one, but many other studies demonstrated the clinical significance of poly-microbial growth from urine sample (Siegman-Igra, 1994). In fact, each of these mixed cultures mostly had at least one of Enterobacteriaceae spp. i.e. *Enterobacter* spp. or *E. coli* or both Table 1. More precisely, out of all these positive-cases, 2 cases had pure gram positive-bacterial infection (8%), while pure gram negative bacterial infection comprised 48% of them and the rest (44%) with both gram-negatives and gram-positive bacterial infection.

In fact, it was shown that highest percentage of isolated uropathogens in patients (23%) had UTI with no appendicitis and comprised both *E. coli* and *S. aureus* (Mohammed *et al.*, 2017). This result is not in agreement with present study, in which Enterobacteriaceae comprised more than 80% of isolates, as pure or mixed infection. This may refers to the role of colonic bacteria (mainly gram-negative ones) in UTI related with AA. These findings may promote other bacteriological studies to search for an evidence for the origination of uropathogens in UTI from colonic flora, then the correlation between bacteriurea and inflamed appendix (Arnbjörnsson, 1988). In fact, although colonic flora may migrate from part of the body across the walls to the lymph nodes and so to the second sites (bacterial translocation) (Salemis, 2009). But, anatomical site plays an additional value here, because appendix is close to the urogenital tract, which in turn reveals how could AA influences these close-structures of urinary system, mainly the ureter and bladder (Gardikis *et al.*, 2002). This may explain more, why the rise in the occurrence of Enterobacteriaceae as uropathogens in patients suffering from AA. As shown in the Figure 1, AA occur in all age groups, with a significant occurrence in the young groups (P<0,05) comparing with older ages. The peak of reported AA cases was observed in the age group 16-22 years; frequency of cases was 24 (52.2%), consisted of males 15 (32.2%) and females 9 (19.6%). Clearly in both gender, results show that incidence rate of appendicitis is declining

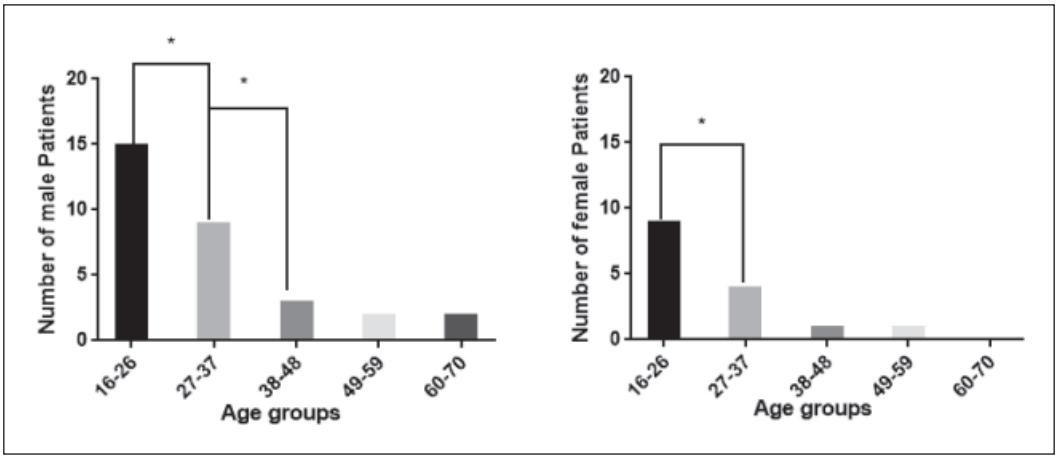

Figure 1. Acute appendicitis distribution by age categories among all 46 patients; A: represent male patients, B: represent female patients. The result were analyzed using one-way ANOVA, *p: <0.05.

with aging factor significantly (P<0.5), with being the lowest rate (4.3%) in groups aged between 60-70. The results are in accordance with other observations being reported previously (Petroianu, 2012; Schick *et al*., 2011). This may due to physiological and anatomical reasons of appendix tissue as described by Jones (Jones, 2001). The lymphoid tissue is the most frequent susceptibility source for infection which gradually increases upto 20 years age then decrease with rising in age up to 60 years old by in which is completely disappeared (Naher and Ktab, 2013). In addition to age factor, incidence rate of acute appendicitis clearly dominant in male 32 (65.3%), in female 14 (34.7%) (Figures 1A and B). This means that males are significantly more susceptible than females with a total ratio 2.2:1. The same results were reported previously (Addiss *et al*., 1990; AL-Fahad, 2003; Naher and Ktab, 2013; Petroianu, 2012). Resistance and sensitivity of the common local used antibiotics for treatment of UTI are reported in Table 3. Microorganisms causing UTIs vary in their susceptibility to antimicrobials from place to place and time to time (Banerjee and Padmashri, 2011). According to National Committee for Clinical Laboratory Standards, 2005 results were assessed as –, Sensitive; as there are a clear inhibition zone; +/–, Partially Sensitive; as the inhibition zone was not clear; +, Resistant; as there are no inhibition zone.

Clinical bacteriological investigations are not complete without an antibiotic sensitivity test of the pathogens on the case-by-case basis. Of the common used antibiotics locally (N=8), *E. coli* showed the highest resistance to these antibiotics except for nitrofurantoin. In fact, data shows that *E. coli* mostly resistance to both ampicillin and gentamycin, following with intermediate zone of inhibition for the rest. Many previous studies demonstrated on the resistance of E.coli to many used antibiotics specifically ciprofloxacin and Sulfamethoxazole/ Trimethoprim (Alanazi *et al*., 2018; Balkhi *et al*., 2018; Sood and Gupta, 2012). These findings elucidate that *E. coli* being the most prevalent during UTIs (Banerjee and Padmashri, 2011). In fact, all isolates showed susceptibility for all these common used antibiotics; ciprofloxacin, trimethoprim/ sulfamethoxazole, amoxicillin-Clavulanic acid and nitrofurantoin, with a superiority of ciprofloxacin and trimethoprim/ sulfamethoxazole. Results can be used to help select the suitable antibiotic drugs that will likely be most effective in treating UTIs.

Conflict of interest disclosure

The authors declare that they have no conflict of interest.

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