

Impact of Some Antibiotics on Bacteria Isolated from Appendices in Kirkuk Province, Iraq

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Abstract

A bacteriological study of 50 appendectomy cases for patients diagnosed by physicians to be appendicitis, within Kirkuk province was carried out to isolate the habitat bacteria of appendix and to assess the impact of some antibiotics on isolated bacterial species from the appendices. The age of patients ranged between 3 - 45 year old (21 male and 29 female). The culture results showed 94% single isolate with 2% negative growth and 4% mixed growth were obtained. The gram positive bacteria isolate formed (11.77%) less than gram negative (88.23%). The total isolates represented 51 included *Escherichia coli* 34 (66.66%), followed by *Enterococcus faecalis* 3 (5.89%), *Klebsiella pneumonia* 3 (5.89%), *Citrobacter youngae* 2 (3.92%), *Raultella terrigena* 2 (3.92%), *Pseudomonas aeruginosa* 2 (3.92%), *Enterobacter cloacae* 1 (1.96%), *Serratia fonticola* 1 (1.96%), *Enterococcus faecium* 1 (1.92%), *Staphylococcus epidermides* 1 (1.96%) and *Staphylococcus xylosus* 1 (1.96%). The *Staphylococcus xylosus* was the first time to be isolated in Kirkuk city while both of *Raultella terrigena* and *Citrobacter youngae* were isolated for the first time in appendix samples. Only 16 various antibiotics were tested against Enterobacteriaceae and *Pseudomonas aeruginosa* while 12 and 7 against *Staphylococcus sp.* and *Enterococcus sp.*, respectively. The Imipenem and Amikacin were found to be most effective antibiotics against all Enterobacteriaceae isolates and *Pseudomonas aeruginosa* while Ampicillin, Piperacillin, Ceftazidime, Augmentin, and Cephalothin were ineffective against all isolated bacteria. The Penicillin and Vancomycin were effective against gram positive bacteria.

Keywords

Bacteria, Antibiotics, Appendix, Appendectomy, Iraq

1. Introduction

The human appendix functions as a reservoir of beneficial microbes that can be

used to recover some clinical events, *i.e.* pathogen colonization, diarrheal disease, or antibiotic treatment [1] [2]. In human, whenever the appendix is infected for any known or unknown reasons, leads to appendicitis, e.g. acute appendicitis or non-acute cases, could cause severe abdominal pain which requires surgical appendectomy as a standard choice of treatment [3] [4]. In some most recent literatures, the acute inflammation is classified as non-complicated appendicitis-inflamed appendix, in the absence of gangrene, perforation, or abscess around the appendix and complicated appendicitis-perforated or gangrenous appendicitis or the presence of peri-appendicular abscess [5] [6]. There are two important components to the pathogenesis of acute appendicitis, *i.e.* obstruction of the appendix and infection by primary pathogenic micro-organism. Bacterial infection is believed to be central to appendix inflammation [7]. Some bacteria may pass the intact appendiceal wall prior to perforation, while progressive infection and subsequent tissue damage with necrosis allows bacteria to move into the peritoneal cavity [8]. Several reports revealed the importance of bacteria particularly *Escherichia coli* and *Bacteroides* spp. in the pathogenesis of appendicitis [9] [10] [11] [12]. The most common organisms present are a mixture of *Escherichia coli*, *Enterococci*, *Anaerobic cocci* together with *Bacteroides fragilis* and *Clostridium perfringens*, which all are normally present in the lumen of the appendix [13]. Recently 32.4% *Escherichia coli*, 18.9% *Bacteroides spp*, 16.2% *Klebsiella pneumoniae*, 9.9% *Pseudomonas aeruginosa*, 6.3% *Citrobacter freundii*, 4.5% *Salmonella typhi*, 4.5% *Proteus mirabilis*, 3.6% *Enterobacter aerogenosa*, 1.8% *Peptostreptococcus*, 0.9% *Staphylococcus aureus* and 0.9% *Clostridium perfringens* bacteria were isolated [14]. Recently, cases of appendicitis had dramatically increased, e.g. 250,000 in USA and 40,000 cases in England are reported each year [15], while a new study in Spain found a rather decrease in appendicitis in the last 10 year [16]. A total of 2375 cases, in year 2013 were reported in public hospitals of Qatar [17].

Due to the lack of bacteriological study of surgically removed appendix within Kirkuk province, this study was performed to isolate various types of bacterial species habitats in the appendix and to assess the effects of various antibiotics on them.

2. Materials and Methods

Fifty sample of appendix were collected from the patients with suspected appendicitis was admitted to the Azadi Hospital, Kirkuk city. Directly, after the operation the samples placed in 1 mL of normal saline 0.9% within a sterile screw-capped container containing. Samples then were transported from the hospital directly to the research laboratory. Inside the hood 1 gram of sample was taken and crushed by a sterile scalpel inside sterile Petri dish and then placed in screw-capped glass tubes contain brain heart infusion broth, the tubes were incubated at 37°C for 24 hrs. Vortex was used to homogenize bacteria in the media, then were cultured onto either MacConkey agar, Blood agar and

Chocolate agar by streaking method. The inoculated plates were incubated aerobically for 24 hour at 37°C followed by examination. When no growth was detected then the plates were re-incubated for another 24 hours before discarding them as negative result. Identification of these bacteria was based on colony morphology on MacConkey agar plates, gram stain and the biochemical test including catalase, oxidase, indole, methyl red, Voges-Proskauer, citrate utilization, motility, and Kiliger Iron Agar (KIA). However, for accurate diagnosis Api 20E (Biomerieux Co.) was used for gram negative identification. The *Staphylococcus* sp. was identified by Vitek2 system and Api Staph (Biomerieux Co.) while the *Enterococcus* sp. was identified by Lancefield grouping (Oxoid, UK) test and Vitek2 system. To assess the potency of various antibiotics for their antimicrobial sensitivity a total of 22 antibiotics were chosen to test using Kirby-Bauer Disc and were compared with those of Clinical and Laboratory Standards Institute [18]. Only 16 antibiotics were used against Enterobacteriaceae and *Pseudomonas aeruginosa* while 12 and 7 were used against *Staphylococcus* sp. and *Enterococcus* sp., respectively. There are some more culturing techniques like anaerobic but, was not employed due to, unfortunately, facility limitation of our laboratories.

3. Results

3.1. Bacterial Isolates

Out of fifty appendices encountered in this project only 47 (94%) specimens were single growth, another specimen (2%) showed negative growth and two specimens (4%) showed mixed growth, e.g. *Klebsiella pneumoniae* with *Enterococcus faecalis* and *Escherichia coli* with *Raoultella terrigena*. The highest percentage of bacteria isolated from the appendix represented *Escherichia coli* 34 (66.66%), followed by *Enterococcus faecalis* 3 (5.89%), *Klebsiella pneumoniae* 3 (5.89%), *Citrobacter youngae* 2 (3.92%), *Raultella terrigena* 2 (3.92%), *Pseudomonas aeruginosa* 2 (3.92%), *Enterobacter cloacae* 1 (1.96%), *Serratia fonticola* 1 (1.96%), *Enterococcus faecium* 1 (1.96%), *Staphylococcus epidermidis* 1 (1.96%) and *Staphylococcus xylosum* 1 (1.96%), respectively (Table 1). The gram positive bacteria represented (11.77%) less than gram negative enteric bacilli which are lactose fermenter or late lactose fermenter except *Pseudomonas aeruginosa*.

3.2. The Antibiotics

The Imipenem (IPM) and Amikacin (AK) were found to be most effective antibiotics against *E. Coli*, *K. pneumoniae*, *E. cloacae*, *C. youngae*, *R. terrigena*, *S. fonticola* and *P. aeruginosa* while Ampicillin (AM), Piperacillin (PRL), Ceftazidime (CAZ), Augmentin (AMC), and Cephalothin (CL) were ineffective against all isolate. All isolates of *E. coli* were 100% sensitive to IPM, Tobramycin (TM), AK, but showed a little less sensitivity to Chloramphenicol (C), moderately sensitive to both Ciprofloxacin (CIP) and Gentamicin (CN) and less sensitive to

Table 1. Types of bacteria isolated, frequency and percentage in both male and female.

Gender Bacteria	Male		Female		Total Isolates No.	%
	No.	%	No.	%		
<i>Escherichia Coli</i>	15	44.1	19	55.9	34	66.66
<i>Serratia fonticola</i>	0	0.0	1	100	1	1.96
<i>Pseudomonas aeruginosa</i>	1	50.0	1	50.0	2	3.92
<i>Klebsiella pneumonia</i>	1	33.3	2	66.7	3	5.89
<i>Staphylococcus epidermides</i>	0	0.0	1	100	1	1.96
<i>Enterococcus faecium</i>	0	0.0	1	100	1	1.96
<i>Enterococcus faecalis</i>	0	0.0	3	100	3	5.89
<i>Citrobacter youngae</i>	2	100	0	0.0	2	3.92
<i>Enterobacter cloacae</i>	0	0.0	1	100	1	1.96
<i>Staphylococcus xylosus</i>	1	100	0	0.0	1	1.96
<i>Raoultella terrigena</i>	1	100	1	100	2	3.92
Total	21	42.0	30	58.0	51	100

both Aztreonam (ATM) and Cefixime (CFM) and even lower sensitivity to both Tetracycline (TE) and to Co-trimoxazole (TS). The *Klebsiella pneumoniae* was highly sensitive to IPM, TM, AK, C, CIP, CFM and Cefotaxime (CTX), but moderately sensitive to ATM, CN, TE and TS. Similarly, the *Enterobacter cloaca* (single isolate) was 100% sensitive to IPM, TM, AK, C, CIP, CFM, CTX, ATM, CS and CN. On the other hand, the *Citrobacter youngae* was sensitive to IPM, TM, AK, TE and CIP but resistant to other antibiotics. The bacteria *Raoultella terrigena* was 100% sensitive to IPM, TM, AK, C, CIP, CFM, CTX, ATM, TE and TS but showed 50% sensitivity to CN. Similarly, the *Serratia fonticola* was 100% sensitive to IPM, TM, AK, C, CIP, CFM, CTX, ATM and CN. However, the bacteria *Pseudomonas aeruginosa* showed 100% sensitivity against IPM, AK, C and CIP but 50% against TM (**Table 2**).

Enterococcus faecalis showed high sensitivity against Vancomycin (VA), and Penicillin (P); however, it displayed moderate sensitivity against AM with lesser sensitivity to both Rifampin (RA) and Doxycycline (DO), while it was resistant to Erythromycin (E). Finally, the *Enterococcus faecium* was highly sensitive against VA, P, and C while it showed resistant to others. Both *Staphylococcus epidermides* and *Staphylococcus xylosus* were fully sensitive to VA, P, CN, CIP, C, RA, TE, AK and TS while the *S. xylosus* showed highly sensitivity to Clindamycin (CC); however, the bacteria *S. epidermides* showed resistant to it. The *S. epidermides* was sensitive to TM while *S. xylosus* showed resistant to it (**Table 3**).

4. Discussion

Approximately one thousand different types of microbes coexist harmoniously

Table 2. Antibiotic susceptibility test for Enterobacteriaceae and *Pseudomonas aeruginosa*.

Antibiotics	<i>E. Coli</i> (N = 34)		<i>K. pneumoniae</i> (N = 3)		<i>E. cloacae</i> (N = 1)		<i>C. youngae</i> (N = 2)		<i>R. trrigena</i> (N = 2)		<i>S. fnticola</i> (N = 1)		<i>P. aeuginosa</i> (N = 2)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
AK	34	100	3	100	1	100	2	100	2	100	1	100	2	100
AMC	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ATM	14	41.2	2	66.7	1	100	0	0	2	100	1	100	0	0
CFM	14	41.2	3	100	1	100	0	0	2	100	1	100	0	0
CAZ	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CTX	9	26.5	3	100	1	100	0	0	2	100	1	100	0	0
CIP	26	76.5	3	100	1	100	2	100	2	100	1	100	2	100
C	33	97	3	100	1	100	0	0	2	100	1	100	2	100
CL	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TS	13	38.2	2	66.7	1	100	0	0	2	100	0	0	0	0
CN	26	76.5	2	66.7	1	100	0	0	1	50	1	100	1	50
IPM	34	100	3	100	1	100	2	100	2	100	1	100	2	100
PRL	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TE	12	35.3	2	66.7	0	0	2	100	2	100	0	0	0	0
TM	34	100	3	100	1	100	2	100	2	100	1	100	1	50

Table 3. Antibiotic susceptibility test against *Staphylococcus* sp. and *Enterococcus* sp. (ND) Not done.

Antibiotics	<i>E. faecalis</i> (N = 3)		<i>E. faecium</i> (N = 1)		<i>S. epidermides</i> (N = 1)		<i>S. xylosus</i> (N = 1)	
	N	%	N	%	N	%	N	%
AK	ND	-	ND	-	1	100	1	100
AM	2	66.7	0	0	ND	-	ND	-
C	3	100	1	100	1	100	1	100
CC	ND	-	ND	-	0	0	1	100
CIP	ND	-	ND	-	1	100	1	100
CN	ND	-	ND	-	1	100	1	100
DO	1	33.3	0	0	ND	-	ND	-
E	0	0	0	0	0	0	0	0
P	3	100	1	100	1	100	1	100
RA	1	33.3	0	0	1	100	1	100
TE	ND	-	ND	-	1	100	1	100
TM	ND	-	ND	-	1	100	0	0
TS	ND	-	ND	-	1	100	1	100
VA	3	100	1	100	1	100	1	100

within a typical healthy human's gut including various bacterial strains—both pathogenic (no-friendly) and non-pathogenic (friendly). They inhabit this chal-

lenging but nutrient rich niche have adapted extremely well, where human would struggle to survive without them. Previous studies have suggested that our friendly bugs secrete chemicals that prevent the pathogenic bacteria from taking control [19]. Eleven types of bacteria identified in this study were subject to antimicrobial test using common antibiotics which are prescribed by Clinical and Laboratory Standards Institute [18]. Other species of bacteria were isolated, *i.e.* Bacteroides sp, *Salmonella typhi*, *Peptostreptococcus*, *Clostridium perfringens* bacteria were isolated [14] have not been isolated in the present work. However, due to current limited techniques available in our laboratories only limited number of bacteria was detectable. This study showed that generally all the bacteria isolated from the appendix samples were both gram positive (11.77%) and gram negative (88.23%). The majority of the bacteria species isolated from appendix samples, cultured and identified were found to be *Escherichia coli* which is in agreement with some recent studies [20] [21] [22]. The frequency of *Klebsiella pneumoniae* appeared less in comparison with other similar studies [23] [24]. A single isolate per of *Raoultella terrigena* and *Serratia fonticola*, were found in our study while no previous references had tackled their species in appendices; however, the *Raoultella terrigena* is a rarely isolatable bacterium from clinical specimens [25]. Our work included description of *Citrobacter youngae* with very low proportion; while *Citrobacter (Citrobacter freundii)*, instead, was isolated in another study [26]. This may refer that this species is the first time to be isolated in appendix. The low rate of *Enterobacter cloacae* found in our study was almost similar to other two previous studies, respectively [20] [26].

Amongst the gram positive bacteria species *Staphylococcus* and *Enterococcus* isolated, another species, *e.g.* *Staphylococcus xylosus* has also been isolated with a very low rate may indicate as if it is an incipience isolate found in the city of Kirkuk (Salman, 2017, Personal communication) while the *Staphylococcus epidermidis* described in the present work had a close rate to another study [23]. Out of 50 samples only the *Enterococcus faecalis* and *Enterococcus faecium* were isolated with low proportions in comparison with two recent studies [21] [24].

The implication of the role of all above bacteria isolated from appendicitis is yet dubious with an unclear pathogenicity function so far while the role of other micro-organism might also be expected. Although the isolated bacteria are classified by other bacteriologists as normal flora but they might have been participated in pathogenicity of the appendicitis. Generally, the variations in the ratio and in the type of bacteria isolated in current study in comparison with others could be due to a few reasons, *i.e.* higher number of the specimens encountered in other studies and the methodology of isolation and culturing involved. Our single negative culture may be a consequence of other microorganism, *i.e.* anaerobic bacteria which need a longer anaerobic incubation period to ensure its growth and identification. Therefore, perhaps more samples of appendices are necessary to drive a righteous conclusion on the habitats and role of other micro-organisms with more antibiotics to test for an overall a precise analysis.

Medical experiences postulate that antibiotics used for treatment appendicitis may equally be effective in certain cases of non-ruptured appendicitis [27]. The results showed that both Imipenem and Amikacin are effective antibiotics against all gram negative bacilli isolates. The latter is in concomitant with a recent work where both Imipenem and Amikacin were well effective against gram negative bacilli [28]. The *E. coli* isolates in our specimens showed various degrees of resistance to Ciprofloxacin, Co-trimoxazole, Tetracycline, Gentamicin which is in agreement with a similar study where *E. coli* resisted to these antibiotics with a slight differences [29]. While all stains of *E. coli* showed resistance against Ceftazidime, Augmentin, Ampicillin, Piperacillin, Cephalothin; however, some stains showed resistance against Cefixime, Azteroname, Chloramphenicol and Cefotaxime. Similar results, with a subtle difference, were found elsewhere too [30] [31] [32] [33].

The resistance of some strains of *K. pneumoniae* to Co-trimoxazole, Tetracycline, Gentamicin, Piperacillin, Ceftazidime, Ampicillin, Azteroname, Augmentin Gentamicin and Cephalothin found in our study was in agreement with most recent studies [34] [35] [36] [37]. The resistance of *Enterobacter cloacae* against Piperacillin, Ceftazidime, Ampicillin, Augmentin, Cephalothin was similar to another study in Egypt [31]. Our *Citrobacter youngae*, showed resistance to almost all the antibiotics tested are in concomitant with some recent studies [38] [39] [40]. The *Pseudomonas aeruginosa* was resistance to Piperacillin, Ceftazidime, Ampicillin, Azteroname, Augmentin and Cephalothin [31] [36].

Generally, the gram positive bacteria encountered here showed sensitivity to both antibiotics Penicillin and Vancomycin [41]. However, *Enterococcus faecalis* showed moderate sensitivity to Ampicillin with little sensitive to Rifampin but with subtle variations [26]. The *E. faecalis* showed sensitivity to Doxycycline and resistance to Erythromycin similar to those of Pinheiro and co-workers [42] while our *Staphylococcus epidermidis* was sensitive to Gentamycin, Ciprofloxacin, Chloramphenicol, Rifampin, Amikacin and Tobramycin [43]; and the *Staphylococcus xylosus* showed resistance to Erythromycin; however, both bacteria resisted Erythromycine [44]. The variation in the resistance of bacteria to antibiotics depends upon species to another and strain to another due to different mechanism of action against antibiotics.

5. Conclusion

The frequency of the gram positive habitat bacteria in the appendix were almost 1:4 of the gram negative with *E. coli* as the highest bacteria followed by other species with low proportion. The bacteria *Citrobacter youngae* was more resistant to antibiotics than other described species. The effectiveness of antibiotics, *i.e.* Amikacin, Tobramycin and Imipenem are specific against gram negative bacilli while Penicillin and Vancomycin against gram positive.

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