

Frequency of Bacterial Spectrum in Patients Presenting with Acute Appendicitis

Muhammad Abdullah¹, Muhammad Farhan Saeed^{2*}, Mirza Sijeel Ahmad³,
Ibrahim Zafar Gondal⁴, Ghazanfar Jabbar⁵, Sajjad Dost¹

ABSTRACT

OBJECTIVE: To define the incidence of bacterial spectrum in individuals with acute appendicitis.

METHODOLOGY: This Descriptive cross-sectional study was conducted at the Department of Surgery, Pak Emirates Military Hospital, Rawalpindi, from November 2023 to April 2024. Statistics were obtained from individuals after written approval. A non-probability consecutive sampling technique was used. 145 Patients who presented with acute appendicitis were included. Non-consenting patients, patients with a history of tuberculosis, pneumonia, urinary tract infection and lower abdominal inflammatory conditions other than appendicitis were excluded. Numerical values were expressed as numbers, mean, and standard deviation and qualitative data were expressed as frequency and percentages. Outcome changers were measured by stratification to determine their efficacy, with a p-value of ≤ 0.05 considered substantial. Statistics were analyzed on SPSS version 20

RESULTS: 145 individuals were involved. The mean age was 43.93 ± 4.28 years. 83 (57.2%) were male and 62 (42.8%) were female. Out of 145 patients with acute appendicitis, the bacterial spectrum showed *E. coli* (34.5%), *Staphylococcus aureus* (5.5%), *Pseudomonas aeruginosa* (30.3%), *Klebsiella pneumoniae* (11%), *Streptococcus* (13.8%), *Enterococcus saprophyticus* (33.1%), and *Bacteroides fragilis* (38.6%).

CONCLUSION: The greater rate of isolation of pathogenic bacteria in patients with acute appendicitis supports its pathogenic role. Gram-negative microorganisms are most common in inflammation of the appendix, equating with gram-positive microorganisms.

KEYWORDS: Appendicitis, Bacterial profile, C-reactive protein, Gram-negative bacteria, Appendectomy, *E. Coli*.

INTRODUCTION

Acute appendicitis is the first and foremost cause of acute abdomen in patients presenting to emergencies with a lifetime threat of 8.6% and 6.7% in men and women correspondingly¹. The origin of appendicular inflammation is multifactorial, which usually presents a diagnostic challenge to the surgeons. Diagnosis requires careful history taking and physical examination; however, delay can lead to morbidity and mortality. The majority of patients present with lower abdominal pain, nausea and fever and are tender in the right iliac fossa on palpation. Still, the disease can present atypically in a few cases. There is no conservative management of appendicitis, and it requires prompt diagnosis, which usually involves clinical judgment along with reliable laboratory inflammatory markers and scoring systems. Moreover, the pediatric population, old age, and gender confound the clinical picture, making the diagnosis a

challenge. Appendectomy is the treatment of choice. Appendicitis is usually caused by obstruction of the closed loop of the appendix due to lymphatic proliferation in the appendix or obstruction by fecal matter. Obstruction increases the intraluminal pressure, which affects blood flow and causes appendiceal obstruction and ischemi, allowing the disease to translocate and infect, causing appendiceal pain². The disease progression can lead to gangrene or perforation, causing peritonitis and sepsis. Multiple pathogens are responsible for causing appendicitis.

The study of bacterial spectrum in cases of appendicitis can provide better management for preventing perioperative infection or even treating catarrhal appendicitis with antibiotics in some cases. Multiple studies have been done to study the bacterial spectrum in appendicitis. Naher HS 2013⁴ found the frequencies of bacterial species as follows: *E. coli* (32.4%), *Klebsiella pneumoniae* (16.2%), *Pseudomonas aeruginosa* (9.9%), *Streptococcus* (1.8%), *Enterococcus saprophyticus* (3.6%), *Staphylococcus aureus* (0.9%), and *Bacteroides fragilis* (18.9%). Another Korean study showed *E. Coli* (66.7%), *Klebsiella pneumoniae* (6.0%), *Pseudomonas aeruginosa* (5.8%), *Streptococcus* (14.7%), *Enterococcus saprophyticus* (7.7%) and *Staphylococci aureus* (1.4%). Another study showed *E. Coli* (57.8%), *Klebsiella pneumoniae* (6.9%),

¹Combined Military Hospital, Hyderabad, Sindh, Pakistan

²Military Hospital, Bhimber, Azad Kashmir, Pakistan

³Combined Military Hospital, Chor, Sindh, Pakistan

⁴Pak Emirates Military Hospital, Rawalpindi, Pakistan

⁵Combined Military Hospital, Peshawar, Pakistan

Correspondence: farhansaeed1290@gmail.com

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Pseudomonas aerogenosa (1.8%), *Streptococcus* (8.7%), *Enterococcus saprophyticus* (15.3%), *Staphylococcus aureus* (2.2%) and *Bacteroids fragilis* (38.2%)⁵.

The study aims to investigate the occurrence of bacterial infection patterns in acute appendicitis, in light of the prevailing perception, as both local and international data have shown variable prevalence rates⁴⁻⁶. The prevalence of bacterial spectrum varies in line with adjustable demographic, ethnic, socioeconomic, and other factors. Furthermore, the pattern of microbiology differs from one hospital to another, with variable susceptibility patterns to antibiotics. As our hospital is a tertiary care facility, we can offer a more accurate representation of the study. Using data from this study would benefit the development of an effective management strategy, thereby improving care standards.

METHODOLOGY

This descriptive cross-sectional research was conducted at the Department of Surgery, Pak Emirates Military Hospital, Rawalpindi, from November 2023 to April 2024. The prerequisite sample size is 145 cases, based on the least frequent *pseudomonas* of 9.9% with an error of 5% and a confidence level of CI = 95%. This sample size was calculated using the WHO sample size calculator - non-probability consecutive sampling. Patients presenting with acute appendicitis as per the operative definition, aged 20-60 years, and of both genders were included in this study. Non-consenting individuals, patients with a history of tuberculosis or pneumonia, and patients with right iliac fossa pain due to gynaecological and urological causes were excluded from this study. Moreover, individuals having Hepatitis C, B or HIV infection, individuals with hypo or hyperthyroidism, patients with a history of cardiac event, asthma, COPD, history of liver or kidney disease and stroke were also excluded from this research.

This research was initiated after obtaining sanction from the College of Physicians and Surgeons of Pakistan. Individuals presenting with acute appendicitis were incorporated from the Department of Surgery, Pak Emirates Military Hospital, Rawalpindi. The institutional ethical review committee's approval was obtained before the study could begin. Every patient gave their informed consent before being included in the study and having their data used for research purposes. A brief history will be taken regarding demographic data (age, gender, and residence status), co-morbidities (diabetes mellitus type II and hypertension), and the period of symptoms. For each patient, height in meters was measured using a wall-mounted scale, and their weight to the nearest kilogram was measured using a weighing device at the time of admission. The BMI

was then calculated. Patients were examined at the time of admission by the investigator under the supervision of a consultant with ten years of practice, and those patients meeting the criteria for acute appendicitis underwent appendectomy by the same surgeon. The surgeon quickly removed a 1-centimetre rim of tissue from the proximal end of the patient's appendicular specimen after it was severed from the caecum. The material was transferred immediately to sterile glass universal bottles, which were appropriately labelled for transmission to the lab for culture, and then into prepared, sterile Robertson Cooked Meat Medium. To extract aerobic bacteria, a portion of the material was grown aseptically on blood, chocolate, and MacConkey agar, respectively, and then immediately incubated at 37°C for 24 hours. The remaining portion was cultured aseptically on anaerobic agar, blood agar, and chocolate agar, in that order. It was then promptly placed into an anaerobic jar containing controlled organisms and a fixed anaerobic kit. After that, they will spend five days being incubated at 37°C.

Statistics were analyzed on SPSS Version 20. Mean and standard deviations for the quantifiable figures, such as age, height, weight, BMI, and length of signs, were calculated. Frequencies and proportions for the qualitative statistics, such as sex, habitation status, type 2 diabetes mellitus, hypertension, obesity status, and bacterial spectrum (*E. coli*, *Klebsiella*, *Pseudomonas*, *Streptococcus*, *Enterococcus*, *Staphylococcus aureus*, and *Bacteroides*) (Yes/No). Result changers were measured by stratifying for age, sex, habitation status, type 2 diabetes mellitus, hypertension, obesity status, and duration of symptoms to assess the impact of these factors on the variable (bacterial spectrum). In this study, a p-value of ≤ 0.05 is considered statistically significant.

RESULTS

A total of 145 patients presenting with acute appendicitis visiting the Department of Surgery, Pak Emirates Military Hospital, Rawalpindi were included, with the age of individuals ranging from 22 years to 60 years. The mean was 43.93 years with a standard deviation of ± 4.28 . At the same time, the mean intervals for symptoms, BMI, height, and weight in our study were 10.15 ± 3.72 hours, 29.41 ± 3.69 kg/m², 148 ± 9.84 cm, and 75.8 ± 6.34 kg, respectively.

Out of 145 patients 56 patients had *bacteroid fragilis* (38.6%) 50 samples revealed *E. coli* (34.5%) 48 patients has *enterococcus saprophyticus* (33.1%), 44 patients had *pseudomonas aeruginosa* (30.3%) while *Streptococcus* (13.8%), *klebsiella pneumoniae* (11%) *staphylococcus aureus* (5.5%) were revealed in 20, 8 and 6 samples respectively (**Table I**)

The stratification of common bacteria by age is presented in **Tables II to VIII**.

Table I: Common Bacteria

Bacteria	No	Percentage
Bacteroid Fragilis	56	38.6
E.Coli	50	34.5
Enterococcus Saprophyticus	48	33.1
Pseudomonas Aeruginosa	44	30.3
Streptococcus	20	13.8
Klebsiella Pneumoniae	8	11
Staphylococcus Aureus	6	5.5

Table II: E. coli According to Age (n=145)

Age	E. coli		Total
	Yes	No	
20-40	24 (48%)	51 (53.7%)	75 (51.7%)
41-60	26 (52%)	44 (46.3%)	70 (48.3%)
Total	50 (100%)	95 (100%)	145 (100%)
P-value	0.31		

Table III: Staphylococcus Aureus according to age (n=145)

Age	Staphylococcus Aureus		Total
	Yes	No	
20-40	06 (75%)	69 (50.4%)	75(51.7%)
41-60	02 (25%)	68 (49.6%)	70(48.3%)
Total	08(100%)	137 (100%)	145(100%)
P-value	0.16		

Table IV: Pseudomonas aerogenosa according to age (n=145)

Age	Pseudomonas Aerogenosa		Total
	Yes	No	
20-40	22 (50%)	53 (52.5%)	75(51.7%)
41-60	22 (50%)	48 (47.5%)	70(48.3%)
TOTAL	44 (100%)	101 (100%)	145(100%)
P-value	0.46		

Table V: Klebsiella pneumonia according to age (n=145)

Age	Klebsiella Pneumonia		Total
	Yes	No	
20-40	11 (68.8%)	64 (49.6%)	75(51.7%)
41-60	05 (31.2%)	65 (50.4%)	70(48.3%)
Total	16 (100%)	129 (100%)	145(100%)
P-value	0.11		

Table VI: Streptococcus according to age (n=145)

Age Years	Streptococcus		Total
	Yes	No	
20-40	04 (20%)	71 (56.8%)	75(51.7%)
41-60	16 (80%)	54 (43.2%)	70(48.3%)
Total	20 (100%)	125 (100%)	145(100%)
P-value	0.01		

Table VII: Enterococcus saprophyticus according to age (n=145)

Age	Enterococcus Saprophyticus		Total
	Yes	No	
20-40	19 (39.6%)	56 (57.7%)	75(51.7%)
41-60	29 (60.4%)	41 (42.3%)	70(48.3%)
Total	48 (100%)	97 (100%)	145(100%)
P-value	0.03		

Table VIII: Bacteroid fragilis according to age (n=145)

Age	Bacteroid Fragilis		Total
	Yes	No	
20-40	15 (26.8%)	60 (67.4%)	75(51.7%)
41-60	41 (73.2%)	29 (32.6%)	70(48.3%)
Total	56 (100%)	89 (100%)	145(100%)
P-value	0.01		

DISCUSSION

The appendix emerges from the cecum as a blind-ending structure. Although it is typically regarded as a vestigial organ, it may play a role in immunity. One of the most common reasons for an acute abdomen requiring emergency surgery is acute appendicitis. The cause of appendicitis is typically brought on by either impacted fecal matter (also known as a fecalith) or lymphoid hyperplasia within the appendix. Obstruction causes bacterial overgrowth, which in turn leads to an increase in intraluminal pressure. This, in turn, obstructs blood flow, causing edema and infection of the appendix, which leads to inflammation of the appendix. The inflammation becomes gangrenous as the infection worsens.

This research included a total of 145 cases. The mean age in our study was 43.93 ± 4.28 years. 83 (57.2%) were male and 62 (42.8%) were female. Out of 145 patients with acute appendicitis, the bacterial spectrum showed E. coli (34.5%), Staphylococcus aureus (5.5%), Pseudomonas aeruginosa (30.3%), Klebsiella pneumoniae (11%), Streptococcus (13.8%), Enterococcus saprophyticus (33.1%), and Bacteroides fragilis (38.6%). Different pathogens are responsible for the disease. Numerous national and international research studies support these findings.

A study conducted by Abid M 2020⁶ showed that bacteriology in patients with acute appendicitis was as follows: *E. coli* 68%, *Streptococci* 27%, *Pseudomonas* 25%, *Klebsiella* 12%, and *Enterococci* 10%.

The study at Partas Children's Hospital in Greece included a cohort of 72 children (43 Boys and 29 Girls). Forty-two children had uncomplicated appendicitis, and 30 children had complicated appendicitis. *E. coli* was the most common organism in both the complicated and uncomplicated groups (63.1% and 37.5%, respectively)⁷.

The prevalence of bacterial spectrum in cases of appendicitis can also help in improving the non-operative management for acute appendicitis. A systematic review and meta-analysis by Maita S 2020⁸ included data from 16 studies, reporting that non-operative treatment was efficient in 92% of patients.

Khalid AJ 2020⁹ described the bacteriology in appendicitis among the adult population. It was a post-hoc secondary analysis of the multicenter study. All the patients with positive cultures after primary and secondary (failure of medical treatment) appendectomy were included. The study revealed *E. coli* to be the most common pathogen cultured in the samples from primary (46%) and secondary (50%) appendectomies.

Acute appendicitis is also one of the common causes of abdominal pain among the pediatric population. A recent study conducted at Children's Clinical University Hospital, Latvia, showed similar results for the most common causative organism among patients with acute appendicitis¹⁰. *Pseudomonas aeruginosa* was the second most common isolated pathogen. The study also demonstrated that most strains of *E. coli* were susceptible to Amoxicillin/clavulanic acid. In acute complex appendicitis, *pseudomonas aeruginosa* was the most common pathogen. It was resistant to Amoxicillin/ clavulanic acid but susceptible to ceftazidime.

In our study, *E. coli* was the most prevalent pathogen among the patients of acute appendicitis. Understanding the bacterial spectrum can efficiently improve the management of acute appendicitis. In another recent analysis, *E. coli* was the most commonly isolated pathogen among patients with acute appendicitis, followed by *Enterococcus*¹¹. The study also demonstrated the susceptibility of strains of *E. coli* to different antibiotic groups.

In a study conducted by Tamura R 2022¹², which included cultures from non-perforated and perforated appendicitis (608 and 72 cases, respectively), *E. coli* and *Bacteroides* were found to be dominant in both conditions. However, the prevalence of *Pseudomonas aeruginosa*, *Streptococcus anginosus* group, and *Enterococcus* group was significantly higher in perforated appendicitis than in the other group.

In a retrospective study by Yukumi S et al.¹³, 106 cases of appendicitis were examined. Fifty-three cases yielded positive cultures, with 26 strains of *E.*

coli identified. 15% were extended-spectrum beta-lactamase producers and 27% were fluoroquinolone-resistant. All were susceptible to carbapenems and tazobactam/piperacillin, with 59% and 82% susceptibility to Clindamycin and Cefmetazole, respectively.

Naher HS 2013⁴ studied bacterial isolates from inflamed appendicitis of 110 patients with acute appendicitis. Positive bacterial cultures were detected in 90 (81.8%) patients, while 20 (18.2%) patients showed no growth. The aerobic bacteria accounted for 87 (78.4%) isolates, whereas the anaerobic bacteria were only 24 (21.6%) isolates. Gram-negative bacteria were present in 107 (96.4%), while gram-positive bacteria accounted for 4 (3.6%). *Escherichia coli* was the dominant pathogens, since it accounted for 36 (32.4%) of all isolates followed by *Bacteroides* spp. 21 (18.9%), *Klebsiella pneumoniae* 18 (16.2 %), *Pseudomonas aeruginosa* 11 (9.9%), *Citrobacter freundii* 7 (6.3%), *Salmonella typhi* 5 (4.5%), *Proteus mirabilis* 5 (4.5%), *Enterobacter aerogenes* 4 (3.6%), *Peptodtreptococcus* 2 (1.8%), *Staphylococcus aureus* 1 (0.9%) and *Clostridium perfringens* 1 (0.9%). Mixed cultures were detected in 21 cases (a Full presentation of this data is not included in this paper), in which more than one organism was identified. Most of the mixed bacterial isolates were aerobic, with 13 (61.9%) being anaerobic. Among these, *Escherichia coli* was the most common, accounting for 15 (71.4%) of the anaerobic isolates.

Laverde B 2023¹⁴ included 1570 patients in a retrospective cohort study who underwent appendectomy for acute appendicitis. Data regarding the intraoperative swab and its microbiology were collected. An intraoperative swab was collected in 29% of the cohort, and the bacterial isolation rate was 51%. These results were assessed on different outcome parameters (morbidity, infectious morbidity, significant morbidity, re-surgery, and prolonged hospital stay). *E. coli* was associated with a higher morbidity (19 vs. 13%) and contagious morbidity (6 vs. 3%) and with a higher rate of prolonged hospital stay (58 vs. 44%). The second bacterium was *Pseudomonas* spp., which was associated with a higher rate of prolonged hospital stay (61 vs. 44%).

In another study, 415 culture-positive perforated appendicitis cases were analyzed. *Escherichia coli* was the most common pathogen (277/415, 66.7%), followed by *Streptococcus* species (61/415, 14.7%). The susceptibility of *E. coli* to ampicillin, piperacillin/tazobactam, ceftriaxone, cefepime, amikacin, gentamicin, and imipenem was 35.1%, 97.1%, 97.0%, 98.2%, 98.9%, 81.8%, and 100%, respectively. The overall susceptibility of *E. coli* to quinolones (ciprofloxacin or levofloxacin) was 78.7%. During the study period, univariate logistic regression analysis showed a significant decrease in *E. coli* susceptibility to quinolones (OR = 0.91, 95% CI 0.84–0.99, P=0.040)¹⁵.

Bacteroides have also been reported to have many

factors that can cause intestinal infections in three ways: by inducing abscess formation, polymorphonuclear leukocytes (PMNLs) are reduced in phagocytic ability because Bacteroidetes produce capsules and inactivate beta-lactamase. The other organisms, including *K. Pneumoniae*, *Clostridium freundii*, *Salmonella enterica* serovar Typhi, *Proteus mirabilis*, and *Enterococcus faecalis* (or *Enterococcus aerogenes*), have also been identified in association with appendicitis. Although the relationship between these microorganisms and appendicular inflammation is unclear due to the presence of intestinal bacteria (Enterobacteriaceae) that are predominantly found in the intestines, all of which possess virulence factors that enable them to adhere, penetrate, and cause disease. The explanation for the recognition of *Pseudomonas aeruginosa* in appendicular inflammation is attributed to the ability of these bacteria to adhere and strengthen the epithelial tissue, likely through the pili and fimbriae (after the mucus) surrounding the bacteria. *Pseudomonas aeruginosa* also exhibits enzyme- and toxin-producing activity, which enables the bacteria to cause disease.

CONCLUSION

The high isolation of pathogenic bacteria in patients with appendicitis supports its pathogenic role. Gram-negative and Gram-positive bacteria, as well as aerobic and anaerobic bacteria, can cause gastroenteritis. Gram-negative bacteria are more common in appendicitis than Gram-positive bacteria. Local statistics are also crucial in mitigating the risk of mistreatment. Bacterial infection should be updated regularly, as some hospital infections occur. The spread of antibiotic resistance should be addressed through an antibiotic management strategy that includes tapering, switching to oral therapy, rapid transfer and discharge of patients, and short-term antibiotic therapy based on the patient's response to initial treatment.

Ethical Permission: Pak Emirates Military Hospital, Rawalpindi, Pakistan, IRB letter No. A/28.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHORS CONTRIBUTION

Abdullah M: Study Design, analysis and interpretation of data, literature search

Saeed MF: Study design, literature search, drafting, analysis, interpretation of data

Ahmed MS: Substantial contribution to the conception

or design of the work, data collection, drafting
Gondal IZ: Substantial contribution to the conception or design of the work, data collection, drafting
Jabbar G: Data collection, data analysis, data interpretation

Dost S: Substantial contribution to the conception or design of the work, literature search, data collection

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