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Antibiotic resistances evaluation and clinical bacteriological analysis in cases of appendicitis

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Abstract

Appendix is a small, closed tube that is about the size of 9 cm. It attaches to the beginning of our large intestine, where the small and large intestines meet. Appendicitis, an inflammation of appendix is mostly related with bacterial etiological agents. The present study is aimed to study bacterial etiological agent that infected the vermiform appendix. Sterile Swabs were used to collect samples from various abdominal sites in addition to the lump of infected appendix. The clinical samples were collected from 69 patients diagnosed as suspected appendicitis from various hospitals in Surat region. Bacterial etiological agents were isolated and identified by standard microbiological procedures. Aamong 69 samples collected, 62 isolates were identified with predominance of gram negative bacteria (n=39), however *Staphylococcus aureus* found as prime pathogen among all. Most of the gram negative isolates found more resistant to Ofloxacin while gram positives against Gentamycin. 47.8% of MDR and 51% of ESBLs producers were knocking outcomes of our study. Further the detection of biofilm formation capabilities of these isolates were carried out by Tube method and Higher degree of biofilm production observed by tube method in compare to microtiter plate method.

Keywords: Appendicitis, Antimicrobial Resistances, ESBL, Bioflim

Introduction

The appendix is usually referred to as a functionless organ but may play a role in immunity. Acute appendicitis is one of the most Recurrent conditions that leads to emergency abdominal surgery. Appendicitis is due to a closed-loop obstruction of the appendix which is usually due to a foreign body, lymphoid hyperplasia or impacted fecal matter which is referred to as a fecalith. Since the appendix can swell in response to infection spread and appendicitis occurred (Naher H.S. and Ktab F.K. et al., 2013). Many types of bioflim producing Microorganisms are present in the appendicitis. According to the National Institutes of Health, up to 80% of human bacterial infections involve bioflim associated Microorganisms (Kosloske A et al., 2004). The standard treatment for appendicitis is appendectomies (surgical removal of appendix), however antibiotic are also effective treatment for appendicitis in mild cases.

Aim and objectives

Aim of present study was to analyze the presence and virulence profile of bacterial etiological agents from the patients suspected for appendicitis. Thus objectives were,

- To isolate and identify bacterial agents associated with appendicitis with standard microbiological procedures
- To study antimicrobial susceptibility pattern of these isolates
- To find out the prevalence of MDR (Multi Drug Resistant) amongst
- To determine of frequency of ESBLs producers
- To study Inducible Clindamycin Resistance among isolates
- To detect of biofilm formation capabilities of these isolates.

MATERIALS AND METHODS

Collection of Sample

A total 69 clinical samples were collected from patients, who were referred as suspected cases of appendicitis to various hospitals in Surat and diagnosed by surgeons as acute appendicitis. Their ages ranged from 4 to 60 years. The appendix is obtained laparoscopy and portions about 2 cm long of appendix removed at operation. The lumen of each appendix was exposed and the mucosa rubbed with a cotton-wool swab.

Isolation of Microorganisms

The impregnated swabs were inoculated on to one plate of MacConkey and Nutrient agar plate. One of each pair of MacConkey and Nutrient agar plates were incubated in 37°C (Naher H.S. and Ktab F.K. et al., 2013). All plates were examined after 24 hours and a examined for bacterial growth and growth characteristics.

Identification of Microorganisms

Suspension of isolates were prepared and carried out morphological identifications (Gram Staining) and biochemical characterization and further grown on various selective media and differential media to support the identification.

Storage of isolate

Isolates were stored on Nutrient agar Slant at 4˚ as working culture and as glycerol stock as stock culture. .

Antimicrobial Susceptibility Test: (CLSI & WHO, 2011).

The isolates were further tested for their antimicrobial susceptibility towards commonly used antibiotics with known concentration (commercially available) was used. Antibiotic susceptibility tests were performed by Kirby-Bauer disk diffusion method.

Determination of Prevalence of MDR

Multidrug resistance is antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drugs. Such microorganism mostly result into therapeutic trailer an even spread the resistance among other species of bacteria by horizontal gene transfer.

Determination of frequency of ESBLs producers (Jacob &prince 2005 & CLSI 2007)

The worldwide prevalence of extended spectrum beta lactamase producing Enterobacteriaceae is increasing making the need for optimization detection technique. The screening of ESBL producer was done according to criteria recommended by the CLSI guideline. For each test contacting cephalosporin alone (ceftriaxone, cefoperaxone) and in combination with clavulanic acid are applied. The inhibition zone around the cephalosporin disc combined with clavulanic acid is compared with the zone around the disc with the cephalosporin alone. The test is positive if

the inhibition zone diameter is >5mm larger with clavulanic acid than without.

Detection of clindamycin Resistance among *Staphylococcus aureus* isolates

Inducible resistance to clindamycin was tested by 'D test' as per CLSI guidelines. 0.5 McFarland turbidity inoculums of well isolated colony of *Staphylococcus aureus* from the plate incubated previously was prepared and inoculated the Muller Hinton Agar plates. After pre diffusion time of 15 minutes the Clindamycin (CLI) disc (2 µg) and Erythromycin (ER) disc (15µg) discs were placed 15 mm apart edge to edge manually with sterile Forcep. Plates were incubated at 37°C for overnight and the plates were observed for the flattening of zone (D-shaped) around clindamycin in the area between the two discs that indicated inducible clindamycin resistances.

Detection of biofilm formation

Bio film formation capabilities amongst all clinical isolates were detected by two methods: Tube Method(TM) (christensen et al., 1982) and Microtiter Plate (MTP) method (christensen et al 1985)

Results

In the 1st stage of study, isolation and identification of bacterial etiological agents was done by standard microbiological procedures. We identified 62 bacterial isolates were obtained from 69 samples collected from patients (46 males and 23 females) and identification of the isolates were done by phenotypic characterization. (Bergey's manual of determination bacteriology, 9th edition and jean F. Macfaddin, Biochemical test for identification of Medical Bacteria,3rd Edition).

Frequency of bacterial isolates:

In present study gram negative bacteria predominated gram positive. However, gram positive isolate, *Staphylococcus aureus* found as prevalent isolate among all.

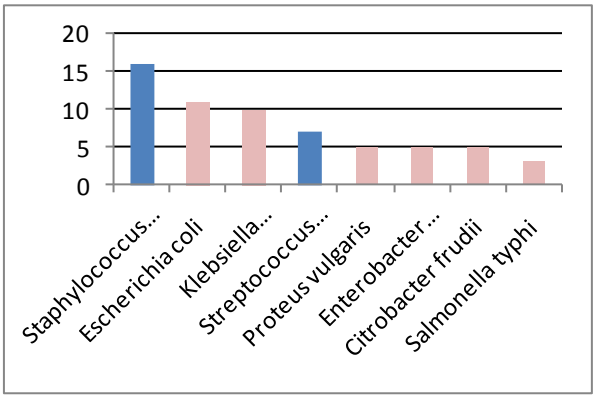

Table-1: Frequency of microorganisms

Result of Antimicrobial susceptibility test

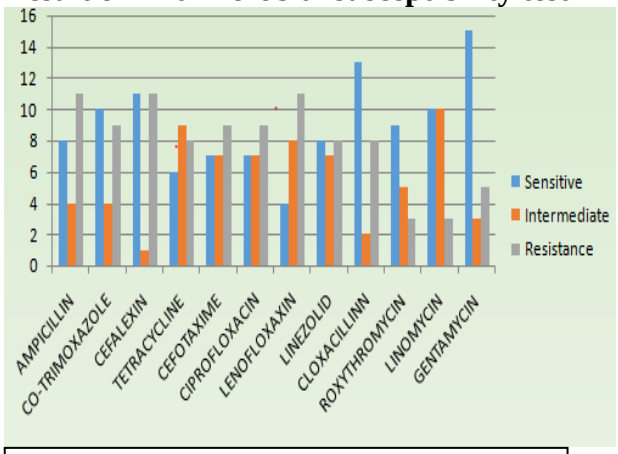

Figure-1 : Antibiogram of Gram Positive isolates

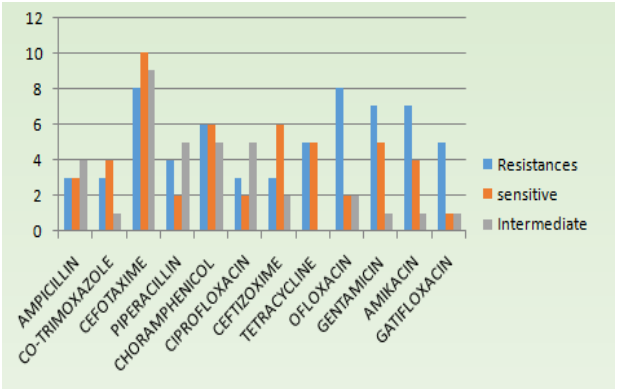

Figure-2: Antibiogram of Gram Negative isolates

Result of MDR (Multidrug resistances)

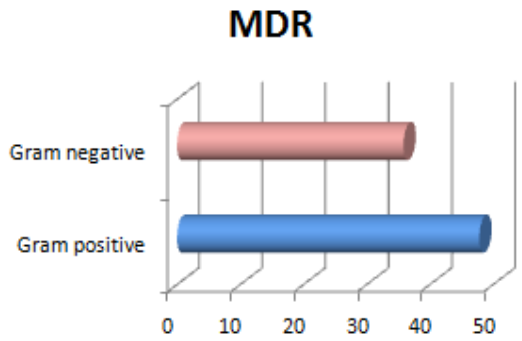

Figure-3 : Multidrug Resistance among the isolates

Result of EsBL producers

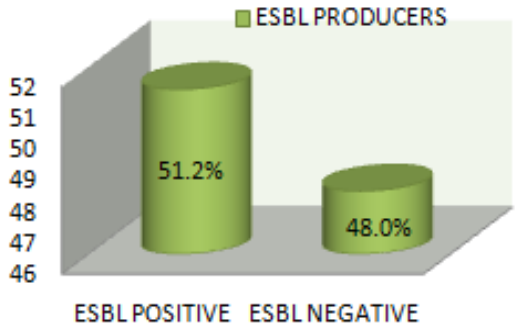

Figure-4: Graphical representation of ESBL producers

Result of D-test

Susceptibility Pattern (phenotype)	No of isolates	Percentage (%)
ERY-S,CL-S	8	50%
ERY-R,CL-R (constitutive, MLS_B)	2	12%
ERY-R,CL-S (D - test positive,iML_B)	5	31%
ERY-R,CL-S (D-test negative, Ms)	1	6%
Total	16	100%

Figure-5: Susceptibility of Erythromycin & Clindamycin among all *S. aureus*

Result of Bioflim formation

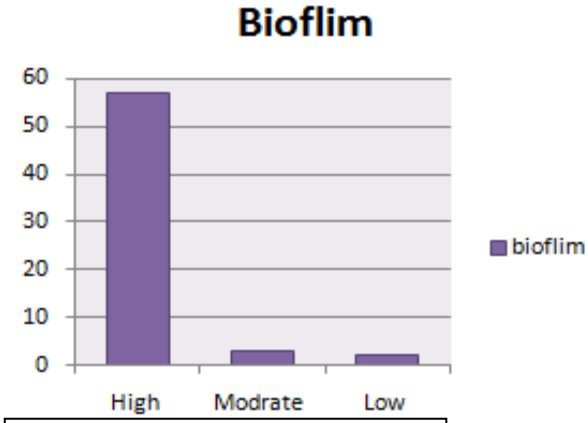

Figure-5: Bioflim formation

Conclusions

This present study was intended to determine the prevalence of bacterial etiogical agents and their virulent role behind human appendicitis. We observed the present of 62 bacterial pathogens obtained from 69 clinical sample of appendicitis. In our study Gram Negative organisms Predominated with high Resistances against Ofloxacin among 51.0% of the isolates found to produce ESBL. And 91.9% bioflim producers associated with appendicitis is the alaming in the patients & knocking the necessity of Microbial analysis.

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