"Isolation, Identification and Antibiotic Susceptibility Pattern of Bacterial Isolates from Fish Sold In Retail Market at Kalyanpur, Kanpur, Up"

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Abstract:

Introduction:-The uncontrolled use of antibiotics increases the emergence of resistant bacteria and makes it difficult to treat the infections. Development of antibiotic resistance in environmental bacteria has become a serious threat and growing problem for the entire population worldwide.

Aim:-To study the microbiological profile and its antibiotic susceptibility pattern from fish sold in retail market at kalyanpur Kanpur, UP.

Material and Methods:-100 Fish Meat and fish intestine sample was collected and preceded under aseptic precaution. Bacterial isolates from the samples on two different bacteriological culture media such as MacConkey agar and blood agar. Mac Conkey agar was used as a selective differential media for differentiating lactose fermenters from non-lactose fermenters .The bacterial isolate were identified by standard bio-chemical tests, Antimicrobial susceptibility testing (AST) was done on Mueller Hinton agar (Hi-Media Laboratories, India) by Kirby Bauer disk diffusion method using Clinical and Laboratory Standard Institute guidelines (CLSI 2020).

Results: - In this study, out of 100 samples 45 were culture positive and 55 were culture negative. Predominant organism was E.coli 31(68%) followed by Proteus mirabilis 11(24%), Klebsiella oxytoca 8 (17%) and Enterococcus 5 (11%). Amikacin, Piperacillin Tazobactam, Polymyxin B and Colistin were 100% sensitive for GNB where as Enterococcus spp was 100% sensitive to HLG, HLS, Vancomycin, Linezolid.

Conclusion: - This study therefore confirms the presence of some pathogenic bacteria isolates from the fish which are of public health significance and indicative of health risk in contacting diseases associated with these organisms. Compliance with standard microbiological measures to prevent contamination by these organisms becomes very necessary and should be enforced. Transfer of antibiotic resistance from these bacteria to pathogenic bacteria can became a serious threat for the human population in the coming future. The usage of high dose of preservatives and antibacterial agents in the fishes during storage can be avoided.

Key Words: Antimicrobial activities, vaginal infection.

Introduction

The intrauterine contraceptive device (IUCD) is one of the most widely used long acting contraceptive methods.[1-3] It offers effective protection from pregnancy and is effective for long-term use. The device can be inserted at anytime as long as pregnancy has been ruled out.[4,5] It is estimated that there are about 180 million users worldwide, with over 80% of these residing in Asia.[6] The use of contraceptives is important in preventing unplanned pregnancy and sexually transmitted diseases. However, the use of various contraceptive methods could expose women to microbial infections. [7] of any contraceptive measure is always observed very keenly and intrauterine device is no exception

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Effect. IUCD is a device, which is fitted into maternal system, evokes a more intent scrutiny. IUCD generally has shown to be safe and effective but tend to have some side effects. [8-18] While female contraceptive methods work differently and affect the quality of vaginal microbial flora both in different population and species variation, hormonal contraceptives such as estrogen and progestogen pills, depo progestin injection, vaginal ring and hormonal IUD prevent pregnancy by interfering with ovulation and suppress the release of an egg from the ovaries[19]. Reproductive tract infection is one of the major complications caused by prolonged usage of an IUCD.[8,11,13,14,18,20,21] It is recognized that use of IUCD results in quantitative and not qualitative changes in vaginal flora. IUCD has been reported to produce inflammation and changes in cervical cytopathology [8, 9, and 17] Different studies have been conducted to explore the IUCD related diseases particularly those associated with infection. Some studies linked the infection related diseases to the insertion method and technique. This is because the post insertion pelvic infection is generally low but appears to be highest in the first 3 weeks' post placement. [13,22] With the

current increase in the level of awareness on reproductive health globally, development and introduction of modern contraceptives, establishment of organized family planning and the desire of families to regulate their family sizes for a more healthy life, more women are increasingly taking to various contraceptive use [23,24].

This study was, therefore, aimed at investigating the microbiological profile and antimicrobial susceptibility of microorganisms isolated from associated with Intra uterine contraceptive users among females of reproductive age.

Material and Methods

Study Setting: This study was being conducted in the Department of Microbiology Rama Medical College Hospital and Research Centre Kanpur.

Samples from outpatients and inpatients admitted to the obs & gynae department, using intra uterine contraceptive devices were collected from Rama Medical college Hospital and Research Centre as the source of the sample for the study.

Study Design: Prospective study.

Type Of Study: Observational study.

Study Period: This study was conducted from January 2020 to December2020.

Size of Sample: 50 sample from patients those using intra uterine contraceptive devices.

Inclusion Criteria: All volunteer women who were presently following Intra uterine device methods, sexually active, ages between 20 and 45 years and were not presently on any antibiotics or having history of antibiotics use three weeks prior the sampling periods were selected.

Excision Criteria: Women with genital infections and contraindications to IUCD insertion such as gynecological cancers, pelvic inflammatory disease (PID) and pregnancy were excluded from the study.

Ethical Consideration: Ethical clearance was taken from the institutional ethical committee.

Sample Collection: High vaginal swab samples were collected. The samples were taken from the cervical canal with sterile cotton swab after cleaning the vaginal area with sterile water and inserting moistened sterile speculum into the cervix. The cotton swabs were gently rotated against the vaginal wall to obtain specimens which were aseptically transferred into the holder, given a numerical labeling before being transferred to the laboratory within two hours of collection for processing. [25]

Direct Smear Gram Stain

Direct smear of the swab samples were prepared on clean glass slides, allowed to air dry and Gram stained using staining method before being examined using oil immersion objective lens (X100) with the condenser iris diaphragm being opened sufficiently to give good contrast.[25]

Wet Preparation Using Normal Saline

The vaginal smears were prepared on clean grease free glass slides and a drop of normal saline was added to each smear, mixed thoroughly and covered with cover glass. The prepared wet smear slides were examined using X10 and X40 objective lens for detection of abnormal cells such as pus cells, white blood cells, epithelia, yeast cells and protozoa before being quantified per high power field.

Bacteriological Examination of Samples

The vaginal swab samples were aseptically streaked on three different culture media including MacConkey agar, Blood Agar and Sabouraud Dextrose Agar. The different agars were prepared according to the manufacturer's instructions following good standard laboratory operating procedures. The prepared agar surface were dried at 45°C for 15 minutes prior to use after which the vaginal swab samples were aseptically streaked onto the different agar plates which were labeled according to the numerical identification numbers earlier assigned to swab samples. All the MacConkey agar plates were incubated at 37°C for 24 hours to produce observable growth colonies. Also, all blood agar plates were incubated at 37°C for 24 hours. Further 24 hours incubation period was allowed for the plates without growth within 24 hours before final results were recorded. Sabouraud dextrose agar plates were incubated at 37°C for 48 hour. Observable colonies on agar plates were subjected to series of bacteriological and biochemical tests according to standard protocol. [26].

Antimicrobial Susceptibility Testing Using Disc Diffusion Method (Kirby-Bauer)

The bacterial inoculate was prepared in 1% sterile peptone water and incubated for 2 hours at 37°C to produce a slight turbidity that was compared with 0.5 McFarland standards. The adjusted inoculate were made into lawn with sterile cotton swabs on dried Mueller-Hinton agar surface. The agar surface was allowed to dry for 15 min before a commercially-prepared Gram negative multi disc containing ciprofloxacin (5 µg), ofloxacin (5 µg), ceftazidime (30 µg), cefuroxime (30 µg), gentamycin (10 µg), amoxicillin/clavulanate (30 µg) and ampicillin (10 µg) ,imipenem(10 µg), meropenem(10 µg),, colistin(10 µg),, polymyxin B(10 µg), was aseptically placed on plates containing Gram negative bacteria while Gram positive multi-disc containing erythromycin (30 µg), cloxacillin (5 µg), gentamycin (10 µg), ceftazidime (30 µg), augmentin (10 µg), vancomycin(30 µg) ,linezolid (30µg),teicoplanin(30µg),ciprofloxacin (5 µg) and tetracycline (10 µg) was aseptically placed on plates containing Gram positive bacteria. The assay was done in duplicate before incubating at 37°C for 24 hours. The inhibition zones formed around each disc were

measured and recorded according to CLSI while the average from duplicate readings was recorded. [27]

[Table/Fig-1]: Social and demographic profile of 50 study population women.

Age (Years)	20-25 (n=10)	26-30 (n=10)	31-35 (n=10)	36-40 (n=10)	41-45 (n=10)
Education background					
a) 10th	-	2	3	5	3
b) 12th	7	3	2	3	2
c) Graduation	3	5	5	2	5
Marital status					
(a) Single	5	4	-	-	-
(b) Married	5	6	10	10	9
(c) Divorcee	-	-	-	-	1
Occupation					
(a) Govt. Job	1	2	2	3	2
(b) Pvt. Job	2	4	2	2	2
(c) Housewife	2	3	6	5	6
(d) Student	5	1	-	-	-
No. of children					
(a) 1-3	2	8	8	8	2
(b) 4-5	-	2	1	1	4
(c) 6 & above	-	-	1	1	4
(d) No child	8	-	-	-	-
Sexual activity per week					
(a) 1-2 times	2	4	7	5	7
(b) 3 & above	8	6	3	5	3

[Table/Fig-2]: Result of the microscopic examination of vaginal swab samples.

Control group	Epithelia cells	Pus cells	Yeast cells	Protozoa *T. vaginalis*
21-25	2	3	1	-
26-30	2	2	1	-
31-35	4	4	3	-
36-40	2	2	2	-
41-45	2	2	2	-
Total	**12**	**13**	**9**	

[Table/Fig-3]: Prevalence of microbes among the Intra uterine devices (IUD) devices.

Age group	No of population study	No of bacteria isolated	No of *candidate* species isolated	No of *T. vaginalis*
Intra uterine devices (IUD)	50 (100%)	4 (8%)	2 (4%)	00 (00%)
20-25	10	-	-	-
26-30	10	-	1	-
31-35	10	1	-	-
36-40	10	2	-	-
41-45	10	1	1	-

Results

[Table/Fig-4]: Result of susceptibility of each bacterial isolate to each of the antibiotics

	Staphylococcus lugdenensis	Staphylococcus epidermis	Escherichia coli	Klebsiella pneumoniae
CIP	S	S	R	R
OF	R	S	R	S
CAZ	R	S	S	S
CEF	R	R	R	S
GEN	S	R	R	R
AMP	R	S	S	S
AMC	R	S	S	R
E	S	S	R	S
CLOX	R	R	R	R
AG	R	R	R	R
STREP	R	R	R	R
CIP	S	S	S	S
TET	R	S	S	S
VAN	S	S	-	-
TEI	S	S	-	-
LZ	S	S	-	-
AZ	S	S	R	R
IMP	S	S	S	S
MRP	S	S	S	S
P	-	-	S	S
CL	-	-	S	S

Discussion

S. No.	Study	Year	Results
1.	**Claudious Gufe et al.**[14]	2019	'This was a cross sectional studies in which a total of 36 fish samples were collected from vendors along shopping centre's in Mufakose, Harare, Zimbabwe, from mid-July to August 2017.
2.	**Chioma M. Ogbukagu* et al**[15]	2021	A total of 720 fishes of Claries gariepinus (African catfish) were aseptically collected from the fish farm sites between the periods of May to October 2016
3.	**In the present study**	2021	Our study showed 100 Fish Meat and fish intestine sample was collected and preceded under aseptic precaution.
1.	**Imarhiagbe, E.E. et al.**[16]	2016	The isolated microbial isolates included Six bacterial and four fungal isolates. They were Staphylococcus spp., Bacillus spp., Pseudomonas spp., Micrococcus spp., Escherichia coli, Corynebacterium spp Aspergillums spp., Fusarium spp., Penicillium spp., and Mucor sp.
2.	**Claudious Gufe et al.**[14]	2019	8 bacteria genera isolated in this study corroborate to 4 bacteria genera isolated from edible fish in Zimbabwe in (*Pseudomonas*, *Escherichia*, *Klebsiella*, and *Proteus*) and to 3 bacteria genera in (*Escherichia*, *Staphylococcus* and *Pseudomonas*). 'is study also corroborates internationally to 3 genera (*Pseudomonas*, *Escherichia*, and *Staphylococcus*) isolated from fish in India.
3.	**In the present study**	2021	Our study showed, E.coli was 31(68%) followed by Proteus mirabilis 11(24%), Klebsiella oxytoca 8 (17%) and Enter ococcus spp 5 (11%).

S. No.	Study	Year	Results
1.	**Imarhiagbe, E.E. et al.**[16]	2016	All the isolates were susceptible to vancomycin, but showed highest resistance to erythromycin (75%). Contrary to our findings, observed a100% resistance to erythromycin from organisms isolated during their work on microbial evaluation and occurrence of antidrug multi-resistant organisms among the indigenous Claries species in River Oluwa, Nigeria
2.	Rahman, M **et al.**[17]	2017	The susceptibility of all the bacterial isolates from fish samples in China were susceptible to vancomycin, cephalexin and florfenicol,ampicillin, chloramphenicol and ciprofloxacin, nitro furantoin, azithromycin, gentamicin and levofloxacin by bacterial isolates from infected fishes.
3.	**In the present study**	2021	Our study showed, Amikacin, Piperacillin Tazobactam, Polymyxin B and Colistin were 100% sensitive for GNB where as Enterococcus spp was 100% sensitive to HLG, HLS, Vancomycin, Linezolid.

Conclusion

This study therefore confirms the presence of some pathogenic bacteria isolates from the fish which are of public health significance and indicative of health risk in contacting diseases associated with these organisms. Compliance with standard microbiological measures to prevent contamination by these organisms becomes very necessary and should be enforced.

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