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Surveillance of multidrug resistant bacteria pathogens from female infertility cases

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Antibiotic resistance is a public health problem of increasing magnitude. Female reproductive system is vulnerable to a number of diseases. Diseased condition results in infertility, menstrual irregularity, pregnancy loss, and in association with pregnancy, morbidity to both the mother and child increases. In the present work, the bacteria pathogens were isolated from the endometrial sample of 50 female infertility cases among which 42 cases were positive for the pathogens. This study reveals that *Escherichia coli* was the most dominant, followed by *Enterococcus faecalis, Pseudomonas aeruginosa*, *Streptococcus* sp., and *Enterobacter*. The isolates were characterized and its susceptibility against important antibiotics were performed. Highest sensitivity was observed with gatifloxacin, imipenam and piperacillin and tazobactum. Thus, according to this study, these antibiotics can be recommended against multi drug resistant bacteria pathogens.

Key words: Multidrug resistance, female infertility, bacteria pathogens.

INTRODUCTION

Nowadays, infertility has become not only a medical, but a social problem as well. One of the most important and underappreciated reproductive health problems in developing countries is the high rate of infertility and childlessness (Bergstrom, 1992; Leke et al., 1993). The female factors contribute most (that is 40 to 55%) in the etiologies of infertility followed by male factors (30 to 40%), both partners (10%) and unexplained factor (10%). There are a number of factors which are responsible for infertility in females. Bacteria infections have long been recognized in association with infertility (Comhaire, 1999; Khalili and Sharifi-Yazdi, 2001) but the significance of these infections in the female and male genital tracts is not well known (Naessons et al., 1986; Golshani et al., 2006). The treatment of bacterial infections is increasingly complicated because of the ability of bacteria to develop resistance to antimicrobial agents. Multi-drug resistant organisms, or MDROs, are bacteria resistant to current antibiotic therapy and therefore difficult to treat. They result in serious local and systemic infections that can be severely debilitating and even life-threatening. The present study reveals the bacterial pathogens in female infertility cases and the antibiotic susceptibility pattern of the isolated bacterial pathogens to determine

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the multidrug resistance pattern.

MATERIALS AND METHODS

Collection of endometrial specimen

The endometrial biopsy tissue was collected by laparoscopic surgical procedure from 50 female infertility cases. An endoscope was inserted into the abdomen through a small incision which gives a complete picture of the endometrium, uterus, etc. Then instruments were threaded through the laparoscope to perform some surgical procedure and finally, the curettage of the endometrium especially from both cornual ends were collected in a sterile saline container, labelled and transported to the laboratory for further analysis. The above samples were collected with the approval institutional ethical committee approval by Bharathidasan University, Tiruchirappalli.

Isolation of bacteria from endometrial specimen of female infertility cases

The tissue samples were collected as explained in the sample collection procedure and further transported to the laboratory in a sterile saline container. Further, it was taken and mechanically digested in a sterile Petri dish to lyse the tissue to get a good microbial quantity for analysis. Blood, Mac Conkey agar, Robertson's cooked meat media was prepared and a loop full of digested tissue was inoculated to the respective culture media. Growth was observed after 24 h incubation at 37°C for bacterial culture.

Morphological characterization

The bacterial isolates obtained from the endometrial specimen were morphologically characterized by Gram staining method.

Biochemical characterization

The organisms which were identified as Gram negative bacilli were inoculated into peptone water for indole production, mannitol motility medium, simmons citrate agar, urease agar, triple sugar iron agar, and methyl red, vogus proskuar broth (MR-VP broth). Oxidase test was also performed. The organisms which were identified as Gram positive cocci were taken for catalase test and coagulase test.

Antibiotic susceptibility test of different bacterial pathogens

The antibiotic susceptibility study was performed for all the isolated bacteria pathogens. Various groups of antibiotics in clinical practice were used in the study to carry out the antibiotic assay against the isolated opportunistic bacterial pathogens. The known quantity of antibiotics was added into nutrient broth which was then inoculated with the bacterial pathogens. The broth with the pathogen alone serves as the control. The set up was incubated for a day at 37°C. The percentage of inhibition of the colonies after the incubation was calculated by plating the cultures from the broth on to the nutrient agar plates and incubating for 24 h at 37°C.

RESULTS

Infertility is on the increase among the Indian population creating stigmatization, and the community as a whole looks down upon such unfortunate couples. In the present study, *E. coli*, *Enterobacter* sp., *Klebsiella*, *Streptococcus*, *Pseudomonas*, *Acinetobacter* sp., and *Staphylococcus* were isolated from the endometrium of the female infertility cases (Table 1) and identified using culture methods. A total of three cases had more than 1 isolate; all the other cases had a single isolate each. There was no chlamydial, gonococcal infection and sexually transmitted disease in the female patients. The isolated bacterial pathogens were characterized biochemically which is illustrated in Table 2.

Table 1. Associated bacterial flora isolated from the endometrium sample of female infertility cases.

Bacteria	Number of Isolates
Escherichia coli	10
Enterobacter	7
Staphylococcus aureus	4
Klebsiella pneumoniae	2
Enterococcus faecalis	8
Pseudomonas aeruginosa	8
Acinetobacter	5
Streptococcus sp.	8

Antimicrobial susceptibility studies of associated bacterial pathogens in female infertility cases

Totally, eight bacteria pathogens were isolated. Among these eight bacteria pathogens, three were Gram positive bacteria and the remaining five were Gram negative bacteria. Antibiotic susceptibility pattern was performed for all the isolated bacteria using the synthetic and semi synthetic group of antibiotics.

In the case of *E. coli*, aminoglycosides groups of antibiotics showed 100% inhibition followed by the fluroquindones class of antibiotics (Table 3). The penicillin group of antibiotics was found to be highly active against all the isolates of *Enterobacter, E. faecalis* and *K. pneumonia*, and *Streptococcus* sp., whereas the same group of antibiotics did not show satisfactory activity against all the clinical isolates of *S. aureus* (Table 3). The amikacin and netilmicin from aminoglycosides showed good activity against all the clinical isolates of gram negative bacilli but not against the *Streptococcus* sp. (Table 3).

The combination of pipercillin and tazobactum, a penicillin antibiotic with extended β– lactamase spectrum, showed 100% percent activity against both Gram positive and Gram negative organisms. These antibiotics are effectively used to treat MDR strain.

DISCUSSION

In this study, a variety of bacteria were isolated from the endometrium of the female infertility cases. Similar study

Table 2. Biochemical characterization of Gram negative bacilli of female infertility cases.

Organism	Motility	Indole	MR	VP	Citrate	Urease	Mannitol	TSI	Oxidase
E. coli	+	+	+	-	-	-	+	A/AG+	-
K. pneumonia	-	-	-	+	+	+	+	K/AG+	-
Enterobacter sp.	-	-	+	-	+	-	+	K/AG+	-
P. aeruginosa	-	-	-	-	+	-	-	K/KG-	+

+ = Positive ; - = negative ; A/A= acid slant/ acid butt ; K/K= alkaline slant/ alkaline butt ; G+= gas positive in sugar fermentation ; G-= gas negative in sugar fermentation.

Table 3. Antibiotic susceptibility testing for the bacterial pathogens isolated from the female infertility cases.

Antibiotic	*E. coli* (%)	*Enterobacter* sp (%)	*S. aureus* (%)	*K. pneumonia* (%)	*E. faecalis* (%)	*P. aeuroginosa* (%)	*Acinetobacter* sp (%)	*Streptococcus* sp (%)
Beta lactam antibiotics								
Amoxycillin	R	R	R	R	100	R	R	100
Amoxyclav	44.40	R	100	100	100	R	60	100
Ampicillin	R	R	R	R	100	R	R	100
Ampicillin+ Sulbactum	R	37.50	100	100	100	R	R	100
Aztreonam	88.90	50	75	100	44.40	R	60	R
Oxacillin	R	R	100	R	33.33	R	R	100
Penicillin	R	R	R	R	100	R	R	100
Piperacillin	44.40	R	R	100	100	100	R	100
Cefoperazone + Sulbactum	100	87.50	R	R	100	100	R	100
Ceftazidime + Clavulanic acid	100	75	R	100	22.20	100	100	R
Piperacillin + Tazobactum	100	100	100	100	100	R	100	100
Cephalosporin antibiotics								
Cefazolin	R	R	100	100	88.90	R	R	100
Cefdinir	55.60	37.50		100	66.70	R	60	100
Cefepime	50	50	100	100	77.80	100	60	100
Cefepirome	66.70	62.50	100	100	88.90	R	80	100
Cefixime	55.60	37.50		100	66.70	R	60	100
Cefoperazone	66.70	50	100	100	77.80	100	80	100

Table 3. Contd.

Ceftazidime	88.30	62.50	R	100	11.11	100	80	100
Ceftizoxime	R	62.50	100	100	77.80	R	80	100
Ceftriaxone	88.90	62.50	100	100	77.80	100	80	100
Cefuroxime	50	37.50	100	100	77.80	R	60	100
Cephalexin	R	R	100	R	R	R	R	R
Cephalothin	R	R	100	R		R	R	R
Cephotaxime	88.30	50	100	100	72.20	100	R	100
Cephalothin /Cephalexin	R	R	R	100	88.90	R	R	100
Fluoroquinolone antibiotics								
Ciprofloxacin	88.90	75	100	100	88.90	100	R	100
Levofloxacin	100	87.50	100	100	100	100	100	100
Lomefloxacin	88.90	75	100	100	88.90	100	80	100
Moxifloxacin	88.90	87.50	100	100	88.90	100	100	100
Norfloxacin	88.90	75	100	100	88.90	100	80	100
Ofloxacin	88.90	87.50	100	100	88.90	100	100	100
Prulifloxacin	88.90	R	100	100	88.90	R	100	100
Sparfloxacin	88.90	75	100	100	88.90	100	100	100
Pefloxacin	88.90	75	100	100	88.90	100	R	100
Gatifloxacin	100	100	100	100	100	100	R	100
Aminoglycoside antibiotics								
Amikacin	100	100	100	100	11.11	100	100	37.50
Gentamicin	R	62.50	100	100	100	100	R	37.50
Netilmicin	100	100	100	100	88.90	100	100	37.50
Tobramycin	88.90	R	R	100	R	100	R	R
Lincosamide antibiotics								
Clindamycin	R	R	100	R	100	R	R	100
Lincomycin	R	R	100	R	100	R	R	100
Glycopeptide antibiotics								
Vancomycin	R	R	100	R	100	R	R	100
Teicoplanin	R	R	100	R	100	R	R	100
Macrolide antibiotics								
Azithromycin	100	75	100	100	100	100	80	100

Table 3. Contd.

Erythromycin	R	R	100	R	55.50	R	R	100
Tetracycline antibiotics								
Doxycycline	77.80	R	100	100	100	R	R	75
Sulfonamide antibiotics								
Co- trimoxazole	R	37.50	75	100	11.11	R	R	R
Quinolone antibiotics								
Nalidixic acid	R	R	R	100	R	R	R	R
Polymyxin antibiotics								
Polymyxin-B	100	100	100	100	100	100	100	R
oxazolidinones antibiotics								
Linezolid	R	R	100	R	100	R	R	100
Nitrofurans antibiotics								
Nitrofurantoin	100	87.50	100	100	100	R	R	100

R, Resistant.

was made on the endometrium of infertile women (Edozien and Akang, 1995). The study reported that high vaginal cultures yielded only bacteria flora similar to fertile women but being complicated by dissemination of quiescent sepsis of the endometrial or vagina. Bacteria infections were detected in 84% of infertility cases. Among the bacterial infections, *E. coli* was the dominant followed by *E. faecalis* and other pathogens (Table 1). A study on the genital infections that were present in infertile couples was done (Rodriguez et al., 2001). A total of 487 patients were diagnosed. Hepatitis was reported in 7.8% cases. *E. coli* is the most prevalent Gram negative organism implicated in urinary tract infection (UTI) (Momoh et al., 2009).

In the present study, *E. coli* was highly sensitive to aminoglycoside group of antibiotics. Aminoglycosides are broad-spectrum antibiotics of high potency that have been traditionally used for the treatment of serious Gram-negative infections (Hermann, 2007). Currently, Amikacin is the most effective of the available antibiotics against many Gram- negative bacteria species (Farrell et al., 2003). Aminoglycosides class of antibioitcs showed 66% sensitivity against *E. coli* followed by the other group of antibiotics (Shyamala et al., 2012). *E. faecalis* was found to be highly sensitive to penicillin group of antibiotics. *E. faecalis* is usually susceptible to penicillin, ampicillin, amoxicillin/clavulanate and imipenem (Metzidie et al., 2006). Kirby was the first person to demonstrate that penicillin was inactivated by penicillin-resistant strains of *S. aureus* (Kirby, 1944). Bondi and Dietz (1945) subsequently identified the specific role of penicillinase. More than 90% of *Staphylococcal* isolates now produce penicillinase.

Aminoglycosides are highly active against *S. aureus* and most clinically significant aerobic Gram-negative bacilli, including *P. aeruginosa* and members of the family Enterobacteriaceae (Weinstein et al., 1964). *P. aeruginosa* and *Acinetobacter* sp., showed high sensitivity to cephalosporins, penicillins and aminoglycoside antibiotics. The imipenem from carbopenum group

showed excellent activity (100%) against both Gram positive and gram negative group of bacteria. Queenan and Bush (2007) states that the carbopenems are the drug of choice for the treatment of serious infections caused by multidrug resistance strains of Enterobacteriaceae producing extended spectrum β – lactamases.

It has been shown that pipercillin and tazobactum are effective against the important Gram positive and Gram negative bacterium especially used in the treatment of nosocomial infection (Sader et al., 1993). Gatifloxacin, antibiotics of the fourth generation belonging to fluoroquinolone family inhibited the bacterial enzymes DNA gyrase and topoisomerase IV. It showed 100% activity on both Gram positive and Gram negative organisms and seems to be efficient in the treatment of MDR strains. But due to the adverse side effects of the antibiotics like liver damage, diabetes, purpura, it has been recently banded by the Union Health and Family Welfare Ministries of India on March 18th 2011. Treatment with appropriate antibiotics for serious bacterial infections would help the society by preventing the development of drug resistance among the bacterial flora.

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