ORIGINAL ARTICLE

Incidence, Isolation and Characterization of Multidrug Resistant Microorganisms Isolated from Urinary Tract Infections of Pregnant Women

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ABSTRACT

Background: The rising resistance of *Pseudomonas aeruginosa, E. coli, Klebisella, Streptococcus* and *Entrococcus* many antimicrobials poses a major therapeutic challenge; Carbapenen-resistant *Pseudomonas aeruginosa*, in particular, exhibit variation in resistance patterns having a great concern in hospitals due to its nosocomial infections.

Aim: To identify the Prevalence of *Pseudomonas aeruginosa*, to compare the outcomes for patients infected with *Pseudomonas aeruginosa* and to look for the antimicrobial activity of antibiotics against *Pseudomonas aeruginosa.*

Study design: Experimental study

Place and duration of study: Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore from 1st March 2017 to 28th February 2018.

Methodology: Fifty urine samples of pregnant women with age range was from 22-45 were studied. Initially, the isolates were identified by colony morphology and different biochemical tests and then by applying Analytical Profile Index (API). Consecutive *Pseudomonas aeruginosa* cultured from different specimens were challenged with locally available antibiotics using the standard disc diffusion method.

Results: The mean age of the women was 46.1 years. There were 18 positive and 32 were no growth. However *E. coli* was seen only in 7 specimens, K*lebisella* in 3, P*esudomonoas* in 2, Streptococcus in 5 and E*ntrococcus* in 1 specimen.

Conclusion: The prevalence of Pseudomonas aeruginosa, E. coli, Klebisella, Pesudomonoas, Streptococcus and Entrococcus were 5% revealed.

Key words: *Pseudomonas aeruginosa*, Nosocomial pathogen, Carbapenem-resistant, *E. coli*, *Klebisella*.

INTRODUCTION

Urinary tract infection (UTI) suggests the presence of microbial pathogens inside the urinary tract. Anything known to be accumulated by the site of disarray (bladder, cystitis), kidney (pyelonephritis) or pee (bacteriuria), UTI can be asymptomatic or symptomatic, delineated by a wide range of responses discharging from smooth irritative voiding into bacteremia, sepsis, or moving through.[1] Urinary tract infection is considered the most observed bacterial disease.[2] Contamination of the urinary tract (UTIs) is arguably the most common bacterial ailments, affecting 150 million people worldwide each year[3].

In all age social relations and in the two sexual forms[4], it has become tendentious. In either case, ladies are more vulnerable than men, considering short urethra, prostatic flood non-appearance, pregnancy, and strong fecal vegetation dirtying of the urinary tract.[5]. In addition, the physiological addition of plasma during pregnancy reduces pee fixation and up to 70% of pregnant women make glucosurea which supports bacterial progression in the pee.[6] Pregnant women are at an increased risk for UTIs. Starting at week 6 and cresting around weeks 22 to 24, about 90 percent of pregnant women undergo urethral dilation, which remains before development. Expanded bladder volume and decreased bladder tone, nearby decreased urethral tone, increase urinary decency and ureterovesic reflux[7].

The entire structure of the urinary tract in the human body includes normal verdure. Customary greenery is generally referred to as ordinary commensals; it appears to people (exogenous) or (endogens) as significant or contradictory grams of live animals or parasites[8], commensalism in a human host condition can be balanced in terms of antagonism to defilement power, pain, compensation, hereditary characteristics, diet, stress, age or ordinary components[9]. UTIs can be understood by voiding urine, as the genitourinary tract passage opens. Microorganisms that consistently live in the urinary tract can cause disease at any rate not normally cause death[10].

At the present time, in these worth estimations, Sanger sequencing beats the greater part of the new advancements. Accordingly, endeavors are in progress to merge data on Sanger sequencing into 454 progression gatherings to improve the nature of the understanding. This recommends, frustratingly, that it is incredibly hard to consolidate and accumulate Sanger sequencing scrutinizes with different sorts of examines, some advancement has been made right now[11].

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MATERIALS AND METHODS

Fresh 50 urine sample of pregnant women were collected form tertiary care hospital, Lahore. The collected sample were placed in the sterile container and volume 100ml approximately. Samples were proceeding further within one hour of collection.

Basic steps for media preparation as following: Pour 100ml of distilled water in a conical flask. Weigh 2.3g of nutrient agar and add it in the flask containing distilled water. Mix this solution properly. Wrap the mouth of the flask with aluminium outwits and label it properly. Now place this conical flask in the autoclave for 15min at 121ºC temp and 15psi Pa. The pH of fresh media is 6.6. After the sterilization is done, allow the media to cool. When it reaches to the suitable temperature or room temperature pour the media in the sterilized petri plates under highly sterilized lab conditions. Let media solidify. After solidification of media plates apply the collected sample dilution carefully under the aseptic condition. Plates must be labelled properly with the sample number and date on which culture is applied and Place the media plates in incubator (35ºC-37ºC) for 24hrs for proper bacterial growth.

Antibiotics sensitivity test (AST) procedure: By means of disc dissemination scheme, Muller Hinton agar plates were organized and disc loaded by way of antibiotic placed on agar and agar plate contains tested bacteria). Labelled plates and dilution syringes properly. Prepared dilutions according to Mcfurland standard. Put 1µl of this dilution on MHA plates to make a lawn with the help of sterile cotton swabs. Making sure appropriate quantity of bacteria used. Drew disc with the help of sterile forceps and placed at appropriate distance from each other. Plates then incubated for 24hourrs. Next day observed agar plates at specific distance noting the lucid region of embarrassment. Zones of inhibition showed effectiveness of particular antibiotic. Calculated thickness of region of embarrassment with the help of vernier caliper and diameter measured in milimeters.

Procedure for DNA extraction: Pouring 10ml of distal water in a test tube,making number of test tubes according to requirements. Weigh 0.4g of nutrient broth and added into these test tubes. Mixed properly and covered the mouth of test tube with aluminium foil and labelled properly. Then placed these tubes in autoclave for 15min at 121ºC at 15psi Pa. Ensured pH 6.6 of fresh media. After sterilization, allowed media to cool. At suitable temperature, poured the store culture (eppendorf) into test tubes. Incubated for 24Hrs or overnight. Took 1ml culture in Eppendorf and centrifuged at 7000rpm for 5min. After centrifugation supernatant was surplus and pellet saved for further processing. After that TE buffer 400µl was added to 100µl SDS 10% and shake very gently, then added protease K 5µl. Kept in incubator at 37ºC for one hour. Added 500µl phenol chloroform mixer at room temperature for five min. Then centrifuge at 10,000rpm for 10min. Three pellets formed were aqueous pellet, inter pellet, organic pellet. Aqueous pellet transferred to fresh eppendorf for next process discarding rest. Added 500µl potassium acetate/sodium chloride at pH of 5.2 and add 1ml of isopropanol and prochilled. Centrifuge at 10,000rpm for 10min. After that supernatant discarded and pellet saved for further processing. Washed with 70% ethanol. Centrifuged at 5,000rpm for 5min and added injection water 25µl in each eppendorf.

Molecular characterization: Molecular characterization means describing at sub-atomic level with no impact of condition or advancement or physiological condition of the life form. That is the reason DNA based markers are called sub-atomic markers and utilizing these sub-atomic portrayals.

Sequencing of DNA: DNA sequencing is the way toward deciding the nucleic acid corrosive arrangement the request for nucleotide in DNA. It fuses any system or development that is used to choose the solicitation for the four bases: adenine, guanine, cytosine and thymine.

Chemicals used in laboratory*:* Constituents of media were attained by the following: E. Merck (Darmstadt, Germany), Difco Labs (Detroit Michigan, USA), Scharlau laboratories (France), Panreac Quimica (Barcelona, Spain) and Sigma-Aldrich Chemicals Co. (St. Louis, USA).

Requirements of laboratory apparatus/instruments: Sterile sample container, Sterile cotton swabs, Petri plates (25ml), Flask (200ml-500ml), Beaker (200ml-500ml), Test tubes (20ml), Glass slides, Sterile syringes, Micropipettes, Sterile tips, Eppendorf tubes, Inoculation platinum wire loop, Bunsen burner, Incubator (temp. range 35-37ºC), Hot air oven, Water bath, Autoclave (121ºC, 15psi), Vortex mixer, Weighing balance, Colony counter and Compound microscope, Glass test tubes holder, Glass slides, Laminar flow, Biosafety cabinet level 2, Digital weighing balance.

Media and chemicals required:: General purpose media (nutrient agar), Differential media (Cystine Lactose Electrolyte Deficient agar CLED), Antibiotics specific media (Muller Hinton Agar), 70% and 95% ethanol, Distilled water, Normal saline, Glycerol, Kovac's reagent, Simmons citrate agar, Triple sugar iron agar with indicator, TMPD dihydrochloride, Urease broth base agar, Urea, Distilled water, Gram staining reagents, Standard antibiotics.

Antibiotics used in research work: Antibiotics used were purchased from approved pharmaceutical company. Various antibiotics selected according to their mechanism of action from 5 diverse groups of antibiotics, routinely used against the bacteria isolated from urine sample of pregnant females**.**

Antibiotics used: Amikacin (Grasil) (AK), Aztreonam (ATM), Ampicillin (AMP), Amoxicillin (AML), Amoxicillin/ Clavulanic Acid (AMC), Cefepime (FEP), Ceftazidime (CAZ), Ciprofloxacin (CIP), Gentamycin (CN), Imipenem (TIENAM) (IMP), Levofloxacin (LEV), Meropenem (MEM), Pipercillin (PRL), Tazopip (TZP), Tobramycin (Nebsin) (TOB), Ampicillin sulbactam (SAM), Cefotaxime (CTX), Ceftriaxona (CRO), Cefuroxime (CXM), Fosfomycin (FOS), Nitrofuranton (NF), Tazopip (TZP), Tetracycline (TE), Linezolid (LZD), Sulfamethoxazole (SXT), Vancomycin (VA), Cephradine (CE), Doxycycline (DO), Fusidic Acid (FD), Tigecycline (TGC), Clindamycin (DA), Penicillin (P), Erythromycin (E).

All material needed for sequencing reaction was supplied to Macrogen sequencing company (Seoul, South Korea). Macrogen sequencing company by using ABI 3730X1 DNA sequence (applied biosystem, USA) and the nucleotide sequencing was done by Sanger sequencing technique. Eight reactions were done by this method.

RESULTS

The mean age of the women was 46.1 years. Eighteen were positive and 32 showed no growth. However, *E. coli* was seen only in 7 specimens, *Klebisella* in 3, *Pesudomonoas* in 2, *Streptococcus* in 5 and *Entrococcus* in 1 specimen. On the basis of Gram reaction, it was observed that 6 (12%) isolates were gram positive and 12 (24%) were gram negative.

Table 1: Sample collection and isolated organisms with their morphological characters

Lab No.	Age (yrs)	Organism	AK	AK1
Sm-1	43	*E. coli*	S	S
Sm-3	46	*P. aeroginosa*	R	-
Sm-4	62	*Klebsiella*	S	S
8048	35	*Entrococcus*	-	S
7948	60	*Klebsiella*	S	S
6513	35	*E. coli*	R	R
Sm-9	28	*streptococcus*	-	S
Sm-10	60	*streptococcus*	-	R
Sm-11	22	*streptococcus*	-	R
Sm-12	54	*streptococcus*	S	R
Sm-13	55	*streptococcus*	-	R
Sm-14	65	*E. coli*	-	R
Sm-15	49	*E. coli*	S	R
Sm-16	32	*E. coli*	R	R
8513	35	*E. coli*	R	R
19562	55	*E. coli*	S	S
19888	50	*P. aeroginosa*	S	-
20580	45	*Klebsiella*	S	R

Fig. 1: Negative culture showed with their highest value factor while gram positive showed their lowest value factor in aspects of collected sample

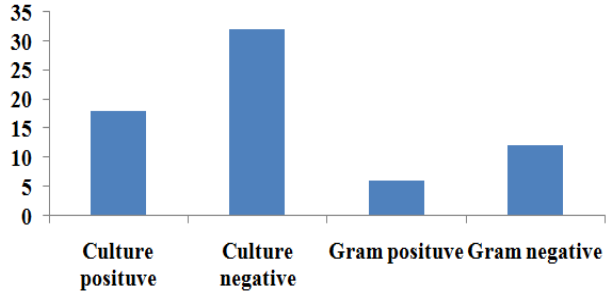

Fig. 2: Colony morphology shown different growth pattern against different agar

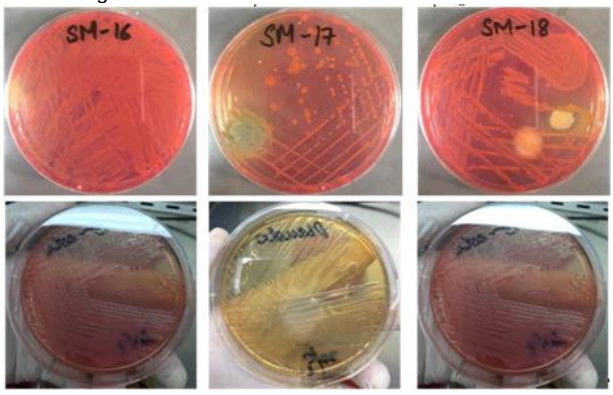

Fig. 2: Antibiotics sensitivity result against different selected bacteria

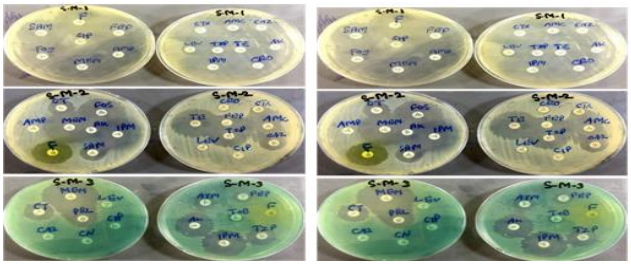

Fig. 3: Conformation of selected DNA bacteria through gel electrophoresis with different sample numbers, showing presence of DNA on given medium. Arrows in gel electrophoresis show value of bp and kb

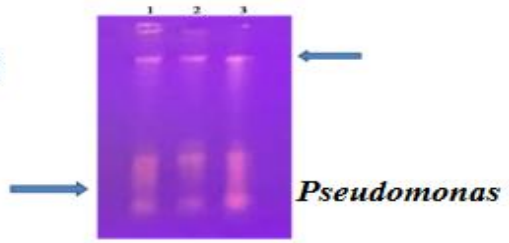

Fig. 4: A trace files analysis shows result of 50 to 90 nucleotides peak of different colors which indicate different pattern. In figure B trace file analysis shows 150 to 190 nucleotides sequence peak which represent different level. Every line search begins with an opportunity (+,-, d, r) descriptor searched for by that occasion's delightful time (like a blast), and from and to focus point, seeing the relationship on which the opportunity occurred.

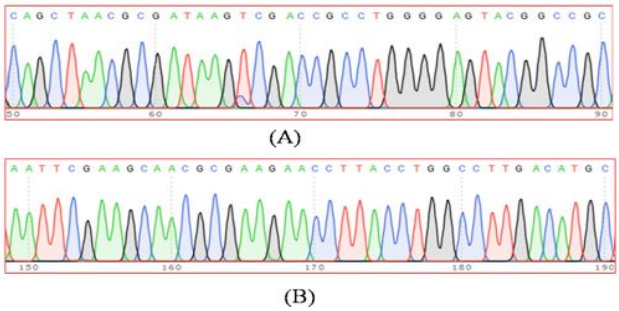

Fig. 5: BLAST is the most commonly used algorithm to execute queries on biological databases. Similar sequences can also be searched by calculating the pair wise alignment score between the query sequence and the database sequences, as per the Smith–Waterman algorithm. For BLAST algorithm, input is given in FASTA format

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GAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGA
GTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCA
GAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGT
GTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGT
CAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTC
AAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAA
GCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCA
ACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTA
GCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGT
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DISCUSSION

The present assessment centered on the nearby status of antimicrobial weakness structure in uropathogens with a

concrete aim of providing assistance in examining the constantly evolving state of bacterial deterrent and further revision of UTI care. It is a sample analysis where standard illustrative results and evaluation of inadequacy are used. The present information have a spot with patients bearing the expense of healing enlistment in a private research place; at this moment, may not duplicate the affirmed amazing quality of UTI as the greater part of the patients were overseen exactly for this contamination.

In our evaluation, Pseudomonas was found to be the second most traditional Gram-negative isolated and had the most significant resistance against ciprofloxacin, levofloxacin, norfloxacin, ofloxacin, and moxifloxacin, while tazobactam/ piperacillin was especially powerless. For the most part *Klebsiella* like *E. coli* (100%) resisted to cephalexin and cephradine, and less opposition to nalidixic damage (20%). A past study from Pakistan reported high affectability (80%) to cefepime versus a continuously undisputed opposition rate (87%) to ciprofloxacin[12]. Nitrofurantoin was the best solution against this pathogen, since it was extremely affectable (100%). Proteus was the least prevalent pathogen and had the most visible square (100 percent) against 12 antibacterial solutions but was found to be 100 percent affectable against meropenem, cefoperazone/sulbactam, and piperacillin/tazobactam, respectively.

In the most recent decade, expanded domain cephalosporin and carbapenem safe Gram-cynical bacilli (GNB) have been extensively revealed in the piece as being dissipated in people yet what's more in creatures and the earth.[13] These ensured creatures regularly cause treatment challenges because of their wide extent of unfriendly to microbial opposition. With the rising of colistin hinder in creatures and its following disclosure in people, the circumstance has expanded. A few assessments bare essential the transmission of safe life structures from creatures to people. Concentrates from within east component the spread of safe life shapes in emergency focuses and to a lesser degree in prepared animals and the earth. In setting on the persistent socio-rational clashes that these nations are looking notwithstanding the tireless masses get together; we attempt right by and by incorporate the holes of the normality of limitation, killing operator poison use reports, disease control measures and other hazard factors contributing expressly to the spread of impediment in these nations. In offices, carbapenemases makers show up, clearly, to be winning. Unusually, extended territory beta lactamases (ESBL) and colistin limitation are changing into a basic issue in creatures. This is commonly a consequence of consistent utilization of colistin in veterinary medicine. In nature, despite the unassuming number of reports, ESBL and carbapenemases makers were both perceived. This features criticalness of the last as a stage among people and creatures in transmission chain. Furthermore, two or three nations are beginning at now going toward issues with untouchables, franticness and poor living conditions which has been obliged by the ordinary war emergency. This all gigantically stimulates the dispersal of limitation in all conditions. In one thriving idea, this work re-features the need to have in general intercession measures to evade dispersal of against microbial limitation in people, creatures and the earth in Middle Eastern nations.

CONCLUSION

On the basis of Gram reaction, it was observed that 6(12%) isolates were gram positive and 12(24%) were gram negative. The current study revealed only 5% Prevalence of *Pseudomonas aeruginosa*, *E. coli*, *Klebisella, Pesudomonoas, Streptococcus* and *Entrococcus.*

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