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ORIGINAL ARTICLE **OPEN ACCESS**

Antimicrobial profiling of *Escherichia coli* and *Pseudomonas aeruginosa* isolated from clinical samples in Nanded city, India

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ABSTRACT

The pathogenic strain of Escherichia coli and Pseudomonas aeruginosa causes nosocomial and community acquired infections in human. β-lactam, aminoglycosides, and fluoroquinolones antibiotics used to treat infections caused by E.coli and P.aeruginosa but the effectiveness of presently available antibiotics decreased due to development of resistance among these pathogenic microorganisms. Therefore, in the present investigation pathogenic microorganisms were isolated from clinical sample and antimicrobial profiling of isolates was analyzed. Two isolates namely SC01 and BL04 were resistant to β-lactam class of antibiotics. On the basis of morphological, biochemical and 16S rRNA gene sequencing, the isolate SC01 SRTMUN and BL04 SRTMUN were identified as Escherichia coli and Pseudomonas aeruginosa respectively.

KEYWORDS: *Clinical samples, Escherichia coli, Pseudomonas aeruginosa, Antibiotic Sensitivity profile.*
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INTRODUCTION:

Human infections such as urinary tract infections, pneumonia, sepsis, meningitis, bloodstream infections, diarrhoea caused by *E.coli* and abscess, corneal infections soft tissue infections, catheter associated infections and conjunctival erythema infections caused by *P.aeruginosa*[1]. *E. coli* and *P. aeruginosa*-related diseases are treated using a variety of antimicrobial treatments[2]. The current antimicrobials including β-lactam antibiotics, aminoglycosides, and fluoroquinolones are considered as significant antimicrobials and considered as crucial chemotherapeutics for humans[3]. Concern that currently available antibiotics won't work against these infections has increased as antibiotic-resistant bacteria have become more frequent[4]. Therefore for effective treatment of diseases caused by *E. coli* and *P. aeruginosa*the proper identification and antimicrobial profile is important. Hence, in the present investigation the antimicrobial profiling of *E. coli* and *P. aeruginosa* strains isolated from clinical sample was analyzed.

MATERIAL AND METHODS

Collection of clinical samples: Skin swabs were collected from the IPD (Indoor Patient Department) of Government Hospital and Medical College, Vishnupuri, Nanded. The swab samples were stored in aseptic collection bulb and transported to the laboratory.

Isolation of pathogens: The collected swabs were inoculated in sterile saline and incubated for 1 hour at 37°C for enrichment of the pathogens associated with it. The enriched inoculums then spread on pre-sterilized nutrient agar plates followed by incubation at 37°C for 24 hours. The well isolated colonies were selected for slanting and used for further identification.

Characterization of isolates: The isolated bacterial colonies were studied for morphological features including size, shape, colour, margin, elevation, opacity, consistency, motility and gram's nature. The biochemical characterization including IMViC test, Catalase, Oxidase and Nitrate Reductase test as well as hydrolysis of starch and urea.

Antibiotic sensitivity profiling: The active culture of isolated bacterial pathogens was spread on nutrient agar plates and incubated for 10 minutes at room temperature (32°C). HiMedia™ antibiotic Octa-disc (Ceftriaxone, Lincomycin, Cefotaxime, Ceftazidime, Netilmicin, Ofloxacin, Vancomycin, and Amikacin) was transferred to the surface of media plate aseptically and plates were incubated at 37°C for 24 hours. After incubation, plates were checked for the zone of inhibition around the antibiotic discs. The zone of inhibition was measured in diameter (mm) using zone measuring scale.

Identification of the isolates: Two isolates namely SC01 and BL04 was identified up to the species level on the basis of 16S rRNA gene sequencing (~1200bp) and phylogeny study. The obtained sequence was blasted on BLAST-NCBI. The most similar sequences were selected for the construction of phylogeny using Mega-X program.

RESULTS

The present investigation is done to isolate the pathogenic microorganisms from the clinical samples and to check their antibiotic sensitivity pattern towards representative presently using antibiotic as therapeutic agents. The bacterial isolates were isolated from the skin swab samples were characterized as gram negative rod shaped bacterium with moderate motility and the biochemical analysis as shown in table.1. In the antibiotic sensitivity profile, many isolates shown resistance towards antibiotics while some antibiotics were capable to inhibit the growth of bacterial population on the agar media (as shown in fig.1 and 2).

The two isolates namely SC01 and BL04 shown resistance to most of the antibiotics and hence selected for the identification using molecular techniques. 16S rRNA gene sequencing up to ~1200bp were carried out and the sequence was checked for the similarities on the NCBI database. The most similar sequences towards SC01 and BL04 were used for the phylogeny construction using maximum likehood method as shown in the fig.3a and 3b respectively and identified as *Escherichia coli* SC01 SRTMUN (NCBI Accession No. OP781965)and *Pseudomonas aeruginosa* BL04 SRTMUN(NCBI Accession No. OP782288).

Table.1 Biochemical characterization of SC01 and BL04 Isolates

Sr. No.	Biochemical test	SC01	BL04
1	**Indole**	+	-
2	**Methyl Red**	+	-
3	**Voges-Proskauer**	-	-
4	**Citrate Utilization**	-	+
5	**Catalase**	+	+
6	**Oxidase**	-	+
7	**Nitrate Reductase**	-	+

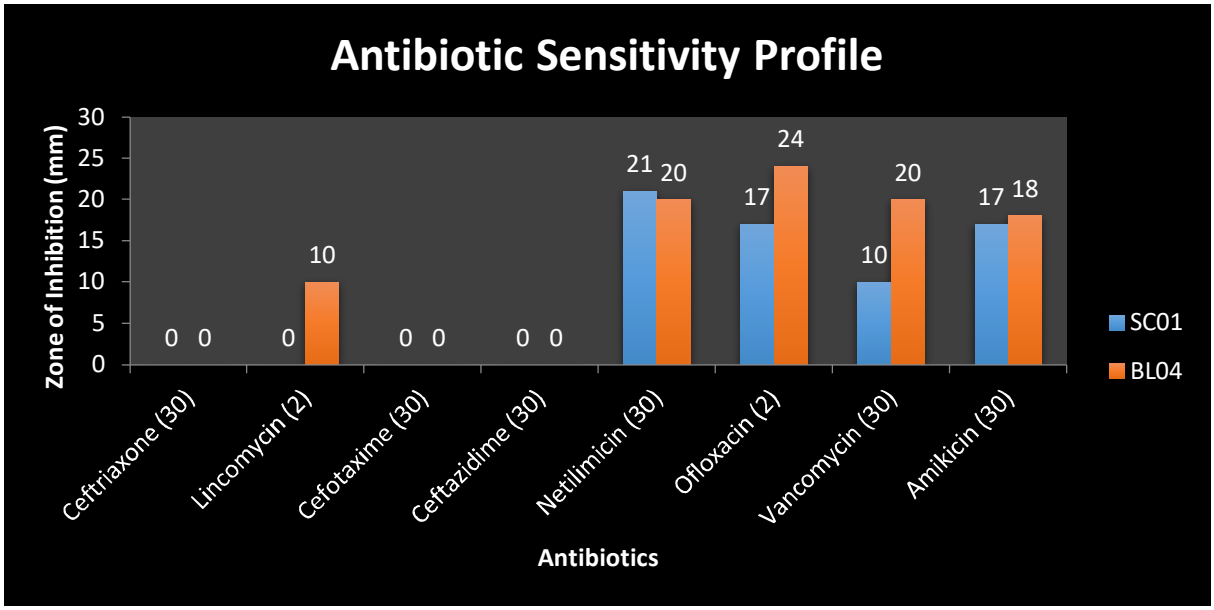

fig.1. Antibiotic Sensitivity Profile of SC01 and BL04

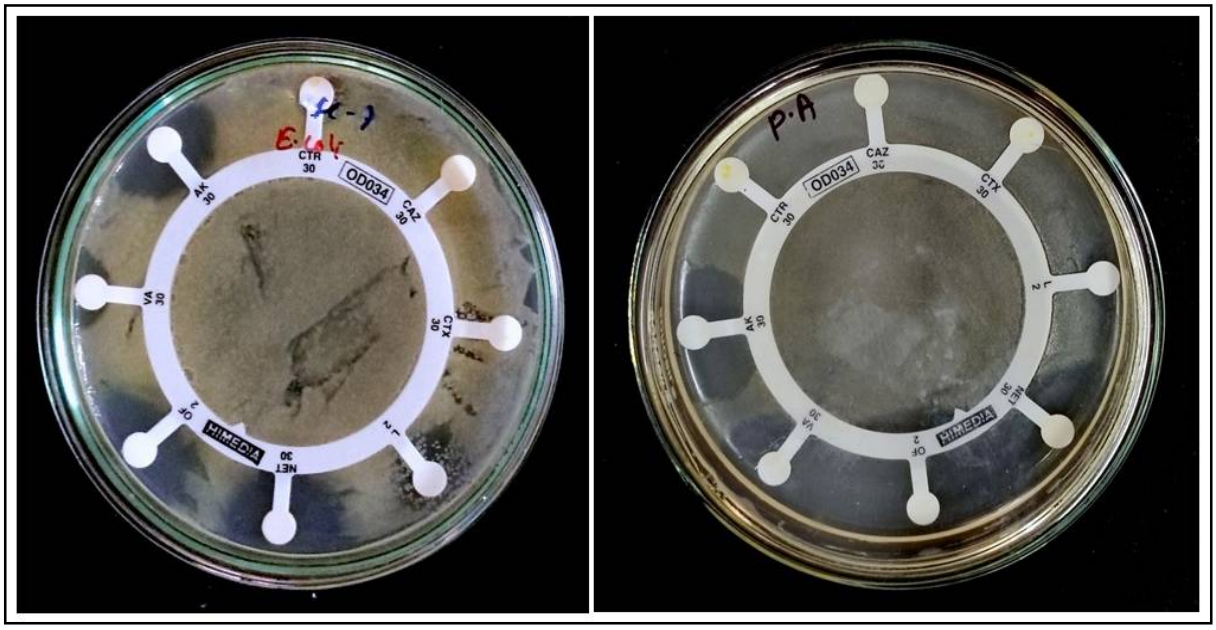

fig. 2. Antibiotic Sensitivity Profile of the isolated pathogens SC01 and BL04

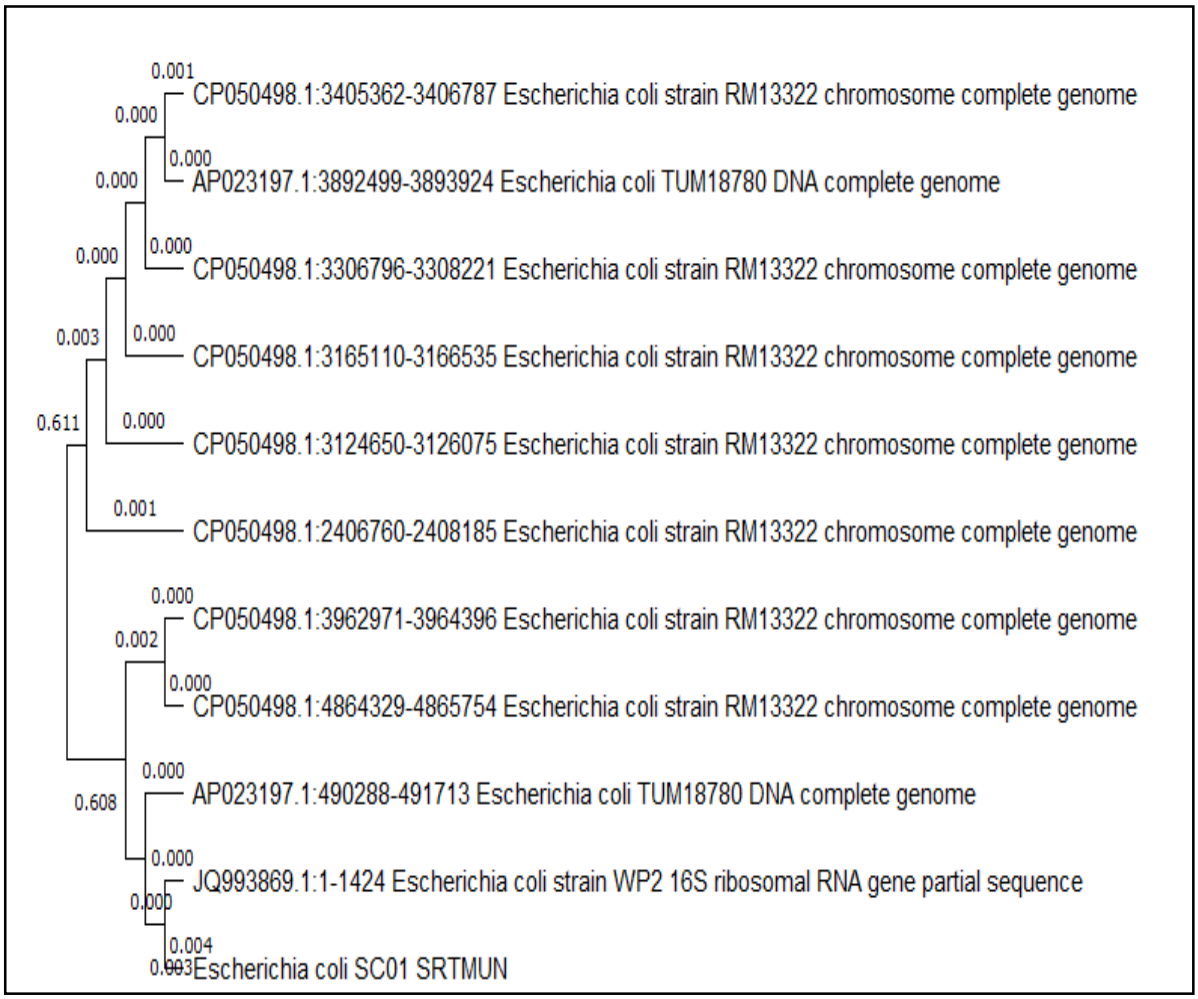

fig.3a. Phylogeny of SC01 isolate identified as *Escherichia coli* SC01 SRTMUN

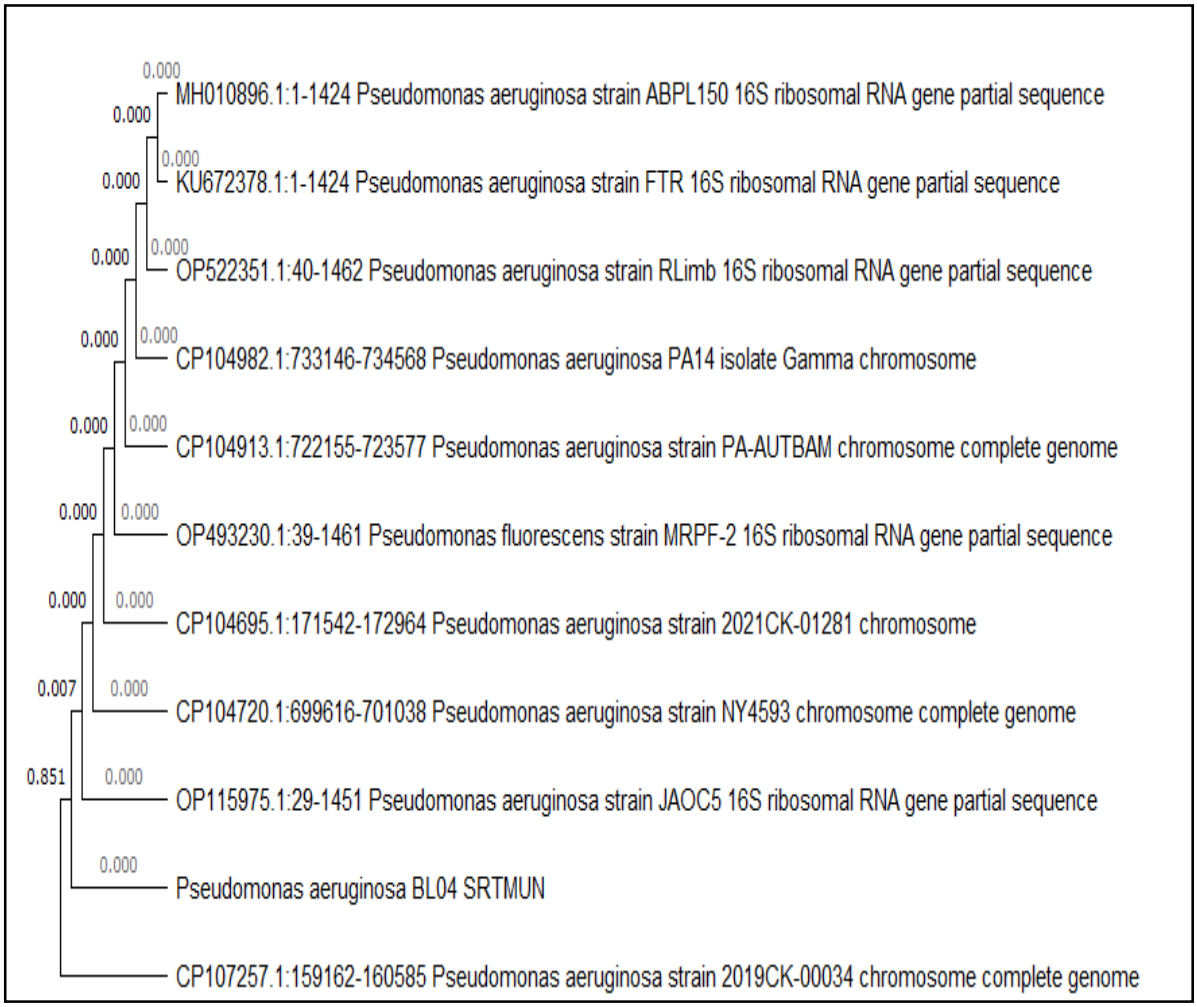

fig.3b. Phylogeny of BL04 isolate identified as *Pseudomonas aeruginosa* BL04 SRTMUN

DISCUSSION

In the present investigation, *Escherichia coli* SC01 SRTMUN isolated from the clinical samples which shown maximum similarities with *Escherichia coli* WP2&*Escherichia coli* TUM18780whereas*Pseudomonas aeruginosa*BL04 SRTMUN shown maximum similarity with *Pseudomonas aeruginosa* strain JAOC5& strain 2019CK-00034. *Escherichia coli* TUM18780 was isolated from patient's wounds of above the pubis and stool samples[5]whereas *Pseudomonas aeruginosa* strain 2019CK-00034 also submitted at Utah Public health laboratory Infectious Disease Submission group[6].*Escherichia coli* SC01 SRTMUN showed sensitivity towards Netilmicin, Ofloxacin, Vancomycin & Amikacin while resistant against Ceftriaxone, Ceftazidime, Cefotaxime & Lincomycin antibiotics. Hongbin et al also studied the antimicrobial profiling of *E. coli* strain using several antibiotic compounds[7]. *Pseudomonas aeruginosa* BL04 SRTMUN shown resistance towards Ceftriaxone, Ceftazidime, Cefotaxime while Lincomycin,Netilmicin, Ofloxacin, Vancomycin & Amikacin antibiotic was shown inhibitory activity of *Pseudomonas* isolate.

CONCLUSION

Two pathogenic bacterial isolates SC01 and BL04 were isolated from clinical samples and identified as *Escherichia coli* SC01 SRTMUN and *Pseudomonas aeruginosa*BL04 SRTMUN. Both the isolates shown antimicrobial resistance against Ceftriaxone, Ceftazidime, Cefotaxime while sensitivity towards Netilmicin, Ofloxacin, Vancomycin and Amikacin. Hence it is concluded that the antimicrobial profiling of the pathogenic microorganism can help before treatment of the diseases.

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