



Case Series of Infections by *Citrobacter* species among COVID 19, Diabetes Mellitus and pulmonary tuberculosis patients at a Tertiary Care Centre from Chengalpattu District, Tamil Nadu, India.

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Submitted: 20-07-2021

Revised: 29-07-2021

Accepted: 31-07-2021

ABSTRACT:

Citrobacter freundii, *Citrobacter koseri* and *Citrobacter amalonaticus* are straight, facultative anaerobic, gram negative, oxidase negative lactose fermenter and non-lactose fermenter bacillus. *Citrobacter freundii*, *Citrobacter koseri* and *Citrobacter amalonaticus* are not frequently associated with infections and isolated from clinical samples among diabetic patients and pulmonary tuberculosis in the Enterobacteriaceae family. The main aim of this case series is to highlight the importance of *Citrobacter* species and the chances of missing out this organism in reporting due to its similar morphology and biochemical reaction which look like KESH (*Klebsiella*, *Enterobacter*, *Serratia* and *Hafnia*) group of organisms. The case series of the organisms are mostly post-operative wound infections, Diabetic foot ulcers, hospital acquired and community acquired pneumonia and urinary tract infections.

Keywords: *Citrobacter freundii*, *Citrobacter koseri*, *Citrobacter amalonaticus*, COVID 19, post-operative wound infection, pneumonia, UTI.

I. INTRODUCTION:

Citrobacter species are straight, facultative anaerobic, gram negative, oxidase negative lactose fermenter and non-lactose fermenter bacillus. Werkman and Gillen in 1932 proposed this genus *Citrobacter* and its species *C. freundii*, *C. diversus* and *C. amalonaticus* [1]. *Citrobacter freundii*, *Citrobacter koseri* (*Citrobacter diversus*) and *Citrobacter amalonaticus* were the three species recognized before 1993. The judicial commission of the International committee on Systemic Bacteriology in 1993 decided to rename *C. diversus* as *C. koseri* [2]. It is frequently isolated from water, soil, food and the intestinal tracts of animals and humans. Infections caused by *Citrobacter* are both community and hospital acquired. Among all gram-

negative infections, 0.8% are caused by *Citrobacter* species [3]. Among the Enterobacteriaceae infections *Citrobacter* species contributes about 3 – 6% of the infections in the hospital settings [4,5]. Infections such as urinary tract infection, respiratory tract infection, wound, bone and soft tissue infection and bloodstream infection can be caused by *Citrobacter* species among the Enterobacteriaceae [5]. Transmission of *Citrobacter* infection may be vertical, horizontal or can be hospital acquired [6]. Urinary tract infection is the most common infection followed by other infections like peritonitis, soft tissue infection, surgical site infection and pneumonia [7].

Case Series Description:

In a period of one month we isolated *Citrobacter* species that included *Citrobacter freundii*, *Citrobacter koseri* and *Citrobacter amalonaticus* from 14 clinical samples. These samples were isolated from diabetic foot ulcers, post-operative wound infections, diabetes mellitus, urinary tract infection, pneumonia in COVID-19 positive and pulmonary tuberculosis patient and puerperal infections in antenatal cases.

Case 1- Associated with Post-Operative Wound Infection And Diabetes Mellitus:

A 58-year-old male presented to general surgery OPD with abdominal pain and swelling over lower abdomen for 1 month duration. The patient was a known case of diabetes and on oral hypoglycaemic drugs. On examination the patient was diagnosed with indirect inguinal hernia over right side. He was admitted and all pre-operative screening investigations like HBsAg, HCV, HIV antibodies and general routine complete blood counts were done. HBsAg, HCV, HIV tests were negative, complete blood counts were within normal limit and blood sugar level was in normal range. His RT-PCR test for COVID-19 was negative. So, patient posted for herniorrhaphy and



procedure was done successfully. After surgery, day 1 and 2 was uneventful, on day 3 there was mild wound gapping with discharge over the operated site. Sutures were removed and wound swab was sent for culture and antibiotic susceptibility testing. *Citrobacter koseri* was isolated from wound swab and it was sensitive to almost all antibiotics tested.

Case 2- COVID 19 Positive Patient:

A 43-year-old male presented to fever clinic OPD with fever and expectorating cough for 7 days. The patient was a not known case of diabetes and hypertension. The patient was diagnosed as "suspected" COVID 19 and pneumonia. He was admitted and RT-PCR (COVID 19), sputum sample for culture, gram stain, acid fast stain and other routine investigations was done. All blood investigations were within normal range, the patient was started with empirical antibiotics. The sputum sample was negative for acid fast bacilli and gram stain showed numerous pus cells with gram negative bacilli. *Citrobacter koseri* was isolated from sputum culture and was sensitive to almost all antibiotics. After 24 hours the RT-PCR results came positive for COVID-19 and patient was treated for the same as per protocol as a moderate case of COVID 19 along with proper precautions and isolation in COVID 19 ward.

The patient did well with Ceftriaxone, spO₂ monitoring. His vital parameters were stable. After 2 weeks repeat sample was sent for RT PCR, sputum culture and CT chest also done. The RT PCR result came as negative and CT chest was normal and sputum culture showed no growth and patient was stable and discharged.

Case 3- Superadded Infection in Pulmonary Tuberculosis:

A 60-year-old male presented to general medicine OPD with breathlessness and expectorating cough for 2 weeks duration. The patient was a known case of diabetes and on oral hypoglycaemic drugs. On clinical examination of the respiratory system, there was wheeze and crackles, other system examination were within normal limits. The patient was diagnosed as acute bronchitis with secondary pneumonia. He was admitted and sputum sample was sent for culture, gram stain and acid-fast stain and other routine investigations was done. The nasopharyngeal swab was sent for RT-PCR and it turned out to be COVID19 negative. All blood investigations were

within normal range except blood glucose. The patient was started with empirical antibiotics and insulin. The sputum sample was positive for ZN stain with a grading of (3+). Gram stain showed numerous pus cells with gram negative bacilli. *Citrobacter freundii* was isolated from sputum culture and was sensitive to almost all antibiotics tested. Anti-tuberculous therapy was started along with Cefotaxime for *Citrobacter freundii* and referred to Sanatorium hospital for further management.

Case 4- Puerperal Infection:

A 25-year-old primigravida presented to Obstetrics OPD for routine antenatal check-up in her third trimester. She was under follow up since conceiving and took necessary investigations, followed all advices and prophylaxis. Now she presented with labour pain and was admitted and delivered a baby boy weighing 3.2 Kg by normal vaginal delivery. After day one she developed lower abdominal pain and fever. The nasopharyngeal swab was sent for RT-PCR and it turned out to be COVID19 negative. Complete physical and per speculum examination was done and high vaginal swab was collected and sent for culture. Nugent scoring on direct gram stain was done and it showed Gram negative bacilli with pus cells and pure growth of *Citrobacter freundii* was obtained. It was sensitive to all antibiotics and patient was treated with Ceftriaxone. The patient responded well to the given antibiotic and was discharged after 5 days. Both mother and baby are doing well.

Seven Cases of Diabetic Foot Ulcer:

Five male and two female patients presented to general surgery OPD with ulcer, pain and swelling over heel (Fig.1). All patients were known case of diabetes and on oral hypoglycaemic drugs. On examination all patients were diagnosed with trophic ulcer over heel, were admitted and all screening investigations like HBsAg, HCV, HIV antibodies and general routine complete blood counts were done. HBsAg, HCV, HIV were negative and routine blood investigations were within normal limit and blood sugar level was also in control except in three patients. Wound swab was sent for culture and antibiotic susceptibility testing. *Citrobacter koseri* and *Citrobacter freundii* was isolated from wound swabs and was sensitive to almost all antibiotics tested except two, which were multi drug resistant strains.



Fig.1- Diabetic foot trophic and non-healing ulcer with discharging pus.

Three Cases of Urinary Tract Infection:

A 48-year-old and 35-year-old female presented to obstetrics and gynaecology OPD and a 45-year-old male presented to general surgery OPD with abdominal pain, burning micturition and tenderness over lower abdomen for more than one week. All the patients did not have a history of diabetes or hypertension. On examination the patients were diagnosed as having urinary tract infection. Ultrasound of abdomen was advised. HBsAg, HCV, HIV were negative and complete blood counts were within normal limit. Two patients had associated with ureteric stones and hydronephrosis. Urine and blood were sent for culture and antibiotic susceptibility testing.

Citrobacter amalonaticus was isolated from urine culture and sensitive to almost all antibiotics tested and blood cultures were negative.

The colony morphology, biochemical characters and antibiotic susceptibility profile are discussed for all samples together. The samples were processed in blood agar, MacConkey agar and chocolate agar. Growth on blood agar showed 2-3 mm, grey to yellow coloured, raised, dry, wrinkled colonies with regular margin and no haemolysis (Fig.2). Growth on MacConkey agar showed 2-3 mm, raised, smooth non-lactose fermenting and lactose fermenting colonies with regular margin (Fig.2).

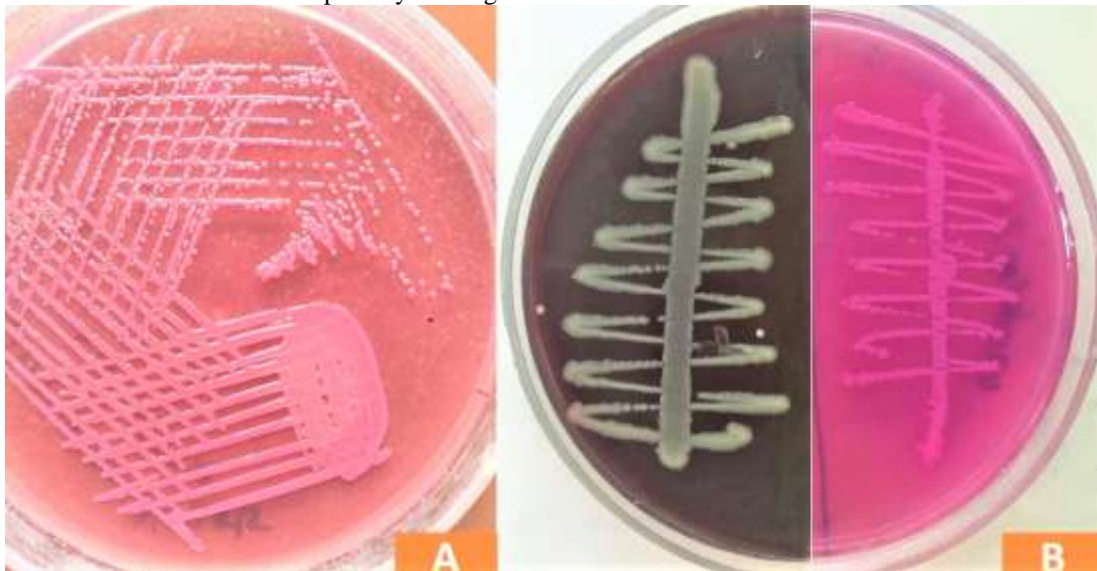


Fig.2- A: Pale non lactose to late lactose fermenting type of colonies of *Citrobacter* species; B- Growth in blood agar and MacConkey agar (Lactose fermenting type)

The Gram stain from the colony showed gram negative bacilli and were catalase positive, oxidase negative and motile. The other biochemical reactions: reduces nitrate to nitrite with gas, citrate

was utilized, urea not hydrolysed, and non-lactose fermenter with variable H₂S in TSI media, indole was positive by *C. koseri* and negative by *C. freundii* and *Citrobacter amalonaticus* (Fig.3).



Fig.3- Various Biochemical and sugar reactions (A & B) for speciation of Citrobacter species.

The antibiotic susceptibility testing was carried out on Mueller Hinton agar with 0.5 McFarland's standard suspension of the colonies with following antibiotics: imipenem, gentamicin, amikacin, ciprofloxacin, cefepime, ceftazidime, cefotaxime, colistin and co-trimoxazole (Fig.4).

The organism was identified as *Citrobacter freundii*, *Citrobacter koseri* and *Citrobacter amalonaticus* with the help of colony characters, biochemical reactions and the isolates were sensitive to all the above antibiotics except co-trimoxazole.

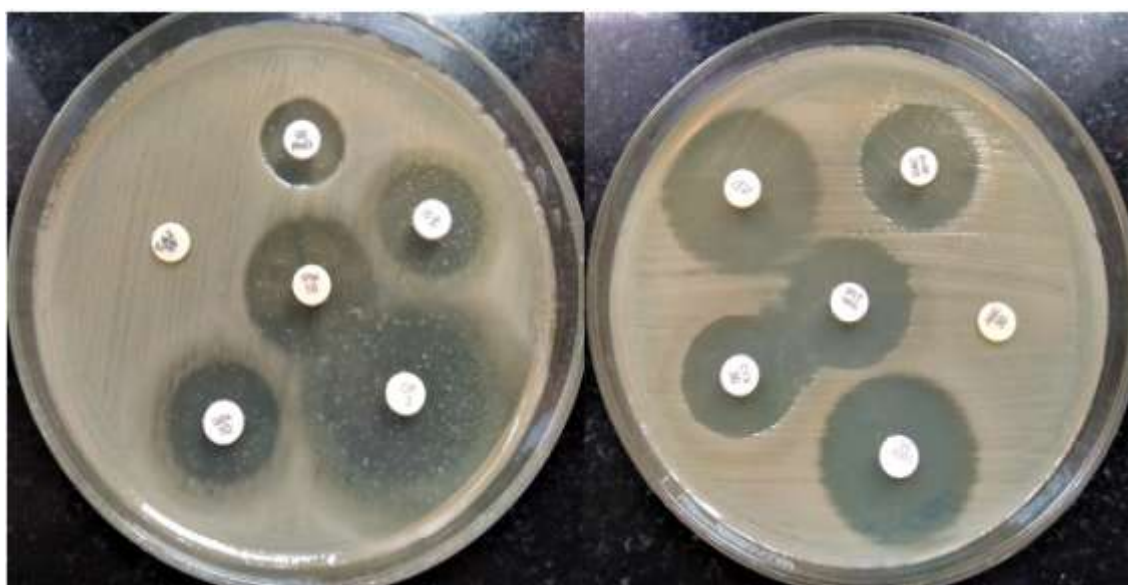


Fig.4- Antimicrobial susceptibility testing by disc diffusion method for Citrobacter species.

II. DISCUSSION:

Citrobacter species are straight, facultative anaerobic, gram negative, oxidase negative lactose fermenter and non-lactose fermenter bacillus. They are frequently isolated from water, soil, food, and the intestinal tracts of animals and humans [1,2]. Infections caused by *Citrobacter* are both community and hospital acquired. Among gram negative infections about 0.8% are caused by *Citrobacter* species. Among the Enterobacteriaceae infections *Citrobacter* species contributes about 3 – 6% in the hospital settings [3,4,5].

Infections such as urinary tract infection, respiratory tract infection, wound, bone and soft tissue infection and bloodstream infection caused by *Citrobacter* species among Enterobacteriaceae. Transmission of *Citrobacter* infection may be vertical or horizontal or hospital acquired. Urinary tract infection is the most common infection followed by other infections like peritonitis, soft tissue infection, surgical site infection and pneumonia [5,6,7]. Onoh RC et al isolated and reported low prevalence of *Citrobacter* species amongst urinary isolates [8]. Colonization of the



urinary bladder was reported by Metri BC and Jyothi P involved in invasive procedures like catheterisation [9]. The host immune system plays a major role in morbidity and mortality of the individuals [9]. The acute infections and urinary tract infections caused by Citrobacter species were reported upto 12% by Tula MY and Whitby et al [9,10].

The main aim of this case series is to highlight the importance of Citrobacter species and the chances of missing out this organism in reporting due to its similar morphology and biochemical reaction which look like KESH (Klebsiella, Enterobacter, Serratia and Hafnia) group of organisms. The case series of the organisms are mostly post-operative wound infections, Diabetic foot ulcers, hospital acquired and community acquired pneumonia and urinary tract infections.

III. CONCLUSION:

Citrobacter is an important member of the Enterobacteriaceae family. It is responsible for causing both community and hospital acquired infections. Many of the hospital acquired infections by this organism may be multi drug resistant. Biochemical and phenotypically it can resemble other members. Hence careful interpretation is very important for its identification. Also, a tab should be kept on its isolation from critical care units so as to identify any outbreak of it these units.

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