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Microbiology

RARE CAUSE OF BACTERAEMIA: MICROBACTERIUM ARBORESCENS.

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ABSTRACT Microbacterium arborescens, among coryneform bacteria are gram positive rods with orange pigmented colonies. Usually these are environmental contaminant but has shown invasive potential in immunocompromised individuals.A 56 yrs old lady presented with uncontrolled hyperglycaemia and hypertension. M. arborescens was isolated on blood culture. Identification was done based on biochemical characterictics and confirmed by Matrix assisted light desorption ionisation Time of Flight Mass Spectrometry(MALDI TOFF MS). Further amplification and sequencing was done and phylogenetic tree was contructed. Tree clearly shows clinical strain PGI 2 is different from environmental strain.

KEYWORDS : Microbacterium arborescens, coryneform, MALDI TOFF MS.

INTRODUCTION:

Among the coryneform bacteria, the phenotypically and phylogenetically closely related genera *Microbacterium* and *Aureobacterium* have been united in the redefined genus *Microbacterium* . At present, the genus *Microbacterium* comprises 55 species , all of which exhibit more or less orange-pigmented gram-positive rods.

Mycobacterium were previously designated as CDC (Centers for Disease Control and Prevention) coryneform groups A-4 and A-5 bacteria. Strains belonging to the genus *Microbacterium* have recently been recognized as pathogens in humans due to an increase in the number of immunocompromised patients, and an increased awareness regarding the pathogenic potential of coryneform bacteria.

CASE REPORT

A 56 years old lady was admitted in department of Endocrinology in a tertiary care hospital in northern India with the complaints of uncontrolled hyperglycaemia along with osmotic symptoms, peripheral vertigo, symptoms of cervical spondylosis and distal peripheral sensory neuropathy. She was a known case of Diabetes Mellites type 2 (DM 2) and systemic hypertension for last 5 years and was taking oral hypoglycaemic drugs and antihypertensive treatment. She was managed with IV fluids, electrolytes and IV insulin through central venous catheter. She was discharged in a satisfactory condition after 7 days of in hospital management.

After 15 days she was again admitted with fever with chills. She was evaluated clinically and aerobic blood culture was sent which were positive for GPB after 48h of incubation. Overnight subcultures on blood agar showed small, orange pigmented colonies Antimicrobial susceptibility by disc diffusion method showed sensitive to amikacin, ampicillin sulbactam, , levofloxacin, clindamycin, Vancomycin , Teicoplanin. She was treated with clindamycin for 7 days. Pt. was without any fever.

Patient again had similar episode of fever with chills after 16 days .Central venous catheter was removed and she received intravenous therapy with vancomycin. Blood cultures were taken again and aerobic cultures in bottles were positive for a GPB as in the previous culture.

Semiquantitative culture of catheter tips was performed by rolling the tip across an agar plate. No growth of any microorganism was detected. The microorganism was identified as M.arborescens by MALDI TOF (Biomerieux) and by 16S ribosomal DNA sequencing.

RESULTS:

The specific characteristics which indicated that the isolate belongs to the genus *Microbacterium* are hydrolysis of gelatin, hydrogen sulphide production, assimilation of malate, citrate,and arabinose. Table 1

Table 1 Characteristic features of Microbacterium arborescens

Charcteristics	M.arborescens
Colour of the colony	Orange
Motility	Positive
Gelatin hydrolysis	Positive
Starch hydrolysis	Negative
H2S production	Positive
VP test	Negative
Arginine dihydrolase	Negative
Arabinose assimilation	Positive
Malate assimilation	Positive
Citrate utilisation	Positive
Propionate assimilation	Negative
Acid from Glucose	Positive
Cell wall diamino acid	Lysine
Major menaquinone acid	MK-11,12

DNA extraction and PCR amplification

DNA was extracted using the overnight bacterial culture using Pure link Genomic DNA Mini Kit (Invitrogen Inc., Carlsbad, CA, USA) according to the manufacturers protocol. Bacterial 16S rRNA gene was amplified using 20 ng of target DNA in a 50 µl PCR reaction mixture.

The PCR mix contained 1X PCR buffer [10 mmol l^{-1} Tris-HCl (pH 8.3), 10 mmol l^{-1} KCl], 2.5 mmol l^{-1} $MgCl_2$, 200 µmol l^{-1} of each dNTP, 200 pmol l^{-1} each forward and reverse primers and 0.5U of Taq polymerase (New England Biolabs). The 16S rRNA gene was amplified using universal primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-TACGGATACCTTGTTACGACTT-3') (Manickam et al, 2010). The PCR cycles included denaturation at 95 C for 5 min, 30 cycles were carried out as follows: 94°C for 30 s, 58°C for 30 s, 72°C for 1 min and final extension at 72°C for 10 min.

Sequencing and Phylogenetic analysis

Purified double stranded PCR fragments were sequenced by chain termination method using ABI Prism Big Dye Terminator cycle sequencing kit (Applied Biosystems Inc., CA, USA) and the samples were analyzed on ABI 3130 XL DNA sequencer (ABI Inc., CA, USA) following manufacturer's instructions[3]. The resulting sequences were assembled using the SEQMANII program (version 4.0; DNAStar Inc., Madison, WI, USA) and analyzed using NCBI BLAST-N.[4] Sequences of closely related matches were retrieved; aligned using ClustalW program and the alignment was manually corrected. For the

neighbour-joining analysis , the distances between the sequences were calculated using Kimura's two-parameter model[5,6]. Bootstrap analysis was performed to assess the confidence limits of the branching [7].

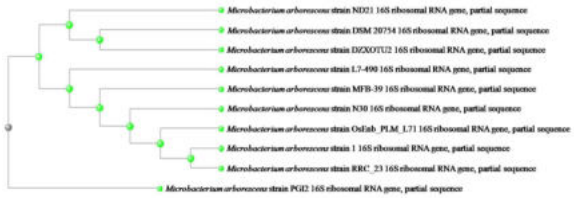

Figure 1: Phylogenetic tree displaying the relationship of nine environmental strains of the M. arborescens and unique clinical isolate of M. arborescen Strain PGI2.

CONCLUSION:

Microbacterium arborescens should be regarded as potential agent of invasive infection. Further studies and case reports are needed to asses true clinical significance of this microorganism.

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