

Atypical Pneumococcal Isolate from Blood

Abstract

Pneumonia with bacteremia accounts for the majority (70%) of cases of invasive pneumococcal diseases. Laboratory characterization of pneumococci is routinely done by sensitivity to optochin. We identified an atypical presentation of optochin-resistant pneumococci in blood isolate of a patient with sepsis. Polymerase chain reaction for autolysin (lytA) and pneumolysin (ply) was positive in the bile soluble isolate. Gene sequencing revealed synonymous single nucleotide polymorphism type of functional mutation in lytA gene. There have been emerging reports of optochin-resistant pneumococci. Diminishing relevance and reliability of optochin sensitivity testing in identifying pneumococci in clinical samples has been highlighted by this case report.

Keywords: Atypical, bacteremia, optochin resistance, pneumococci

Introduction

Isolation of *Streptococcus pneumoniae* from normally sterile sites such as blood, cerebrospinal fluid, synovial fluid, and pleural fluid is the hallmark of invasive pneumococcal infections. Standard biochemical test such as optochin susceptibility with a zone diameter of ≥ 14 mm has been the sole criteria to isolate pneumococci from clinical samples. There have been reports of emerging optochin-resistant pneumococci in a few countries.^[1-3] It was first reported from Finland and sporadic reports of isolates from different geographic areas have since been documented. This report is rare because we identified atypical pneumococci in blood isolate and thereby contributing to better treatment options.

Case Report

We identified one alpha-hemolytic isolate from the blood culture of a 67-year-old male patient admitted to the intensive care unit with suspected sepsis. The Gram stain revealed characteristic lanceolate diplococci, and capsule staining by India ink was positive for pneumococci. Further tests such as optochin sensitivity, bile solubility, and inulin fermentation were done. The isolate was found to be optochin-resistant, bile soluble, and fermented inulin. Identification by both automated systems VITEK II

and MALDI-TOF was *S. pneumoniae*, and detection of lytA gene and ply gene, encoding for virulence factors autolysin and pneumolysin, respectively, were found to be positive [Figure 1]. Further Gene sequencing was done, and the isolate was found to have the synonymous single nucleotide polymorphism type of functional mutation in lytA gene [Figures 2 and 3]. The patient responded well following treatment with ceftriaxone and was discharged after complete recovery.

Discussion

Kellogg *et al.*^[4] in 2001 have reviewed the relevance of optochin susceptibility testing in differentiating between pneumococcal and nonpneumococcal isolates, and have

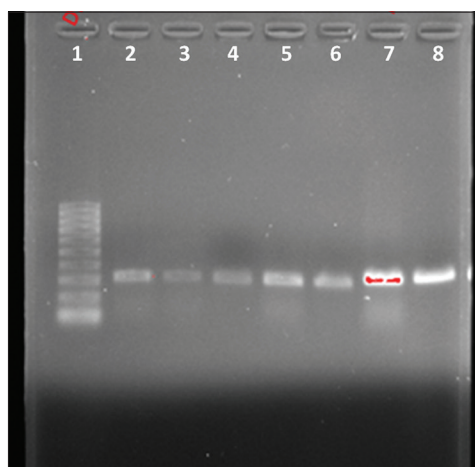


Figure 1: Lane 1 – DNA ladder, lane 2 – lytA (319 bp) and lane 7 – ply gene (348 bp) for optochin-resistant bile soluble blood isolate

How to cite this article: Veerasamy J, Jayarajan J. Atypical pneumococcal isolate from blood. *Int J App Basic Med Res* 2018;8:61-3.

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Received: 11 December, 2016.
Accepted: 02 January, 2018.

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Access this article online

Website:
www.ijabmr.org

DOI:
10.4103/ijabmr.IJABMR_459_16

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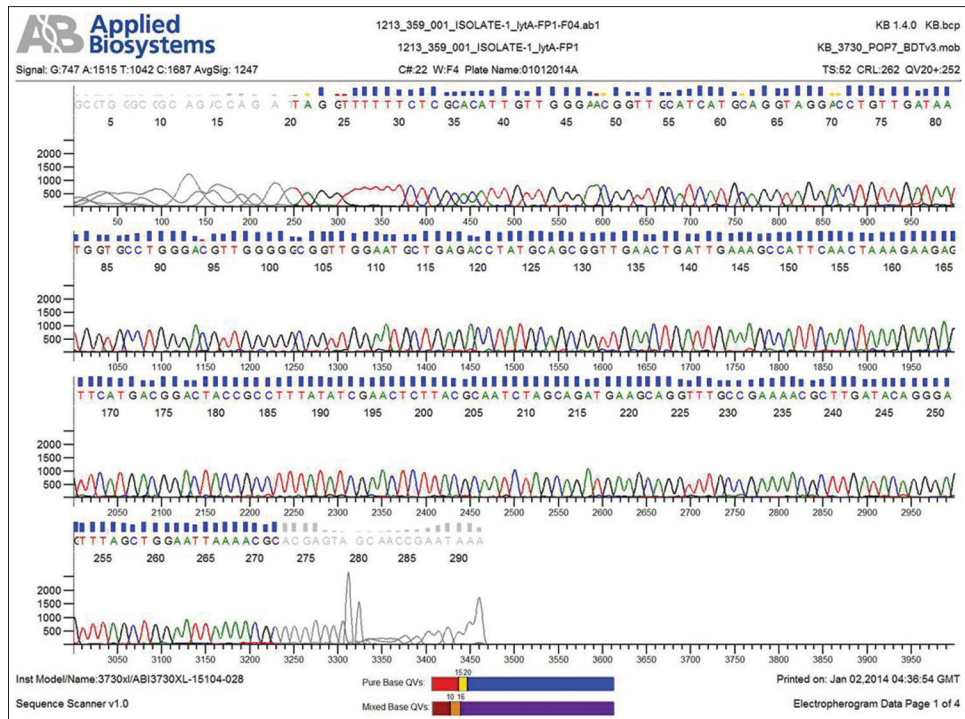


Figure 2: Gene sequencing for lyt A (forward primer)

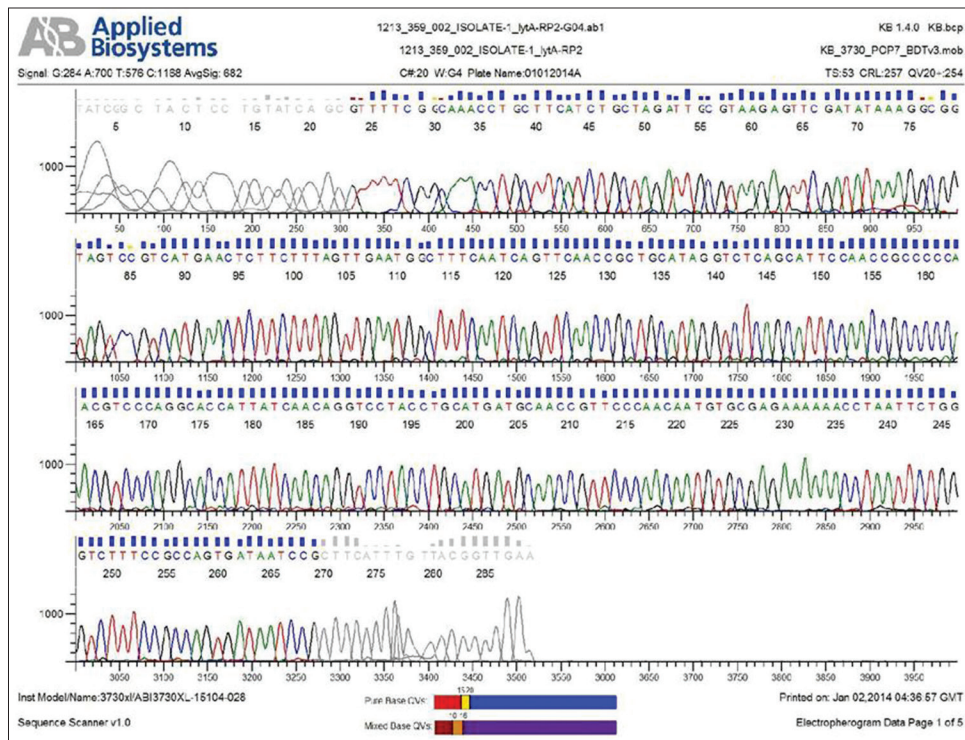


Figure 3: Gene sequencing for lyt A (reverse primer)

concluded that sensitivity and specificity of optochin sensitivity testing was 99% and 98%, respectively. Data collected between 1993 and 1996 regarding pneumococci in few regions of USA by Borek *et al.* have been analyzed and the diminishing relevance of traditional optochin sensitivity testing in accurately identifying *S.*

pneumoniae has been pointed out.^[5] Optochin-resistant pneumococci, bile insoluble *S. pneumoniae* isolates and nontypeable, unencapsulated pneumococci have been reported to comprise 2% of pneumococci isolated from normally sterile sites.^[6] Analysis of genetic and biochemical characterization of optochin sensitivity by

Ferrándiz and de la Campa^[7] (2002) highlighted that typical optochin susceptibility is decided by the nature of the F_0 complex of its $F_0F_1H^+$ ATPase, an enzyme that is essential for the viability of the organism. Characterization of optochin-sensitive viridians streptococci by Martín-Galiano *et al.* from Spain (2003) have revealed the acquisition of *atp C*, *atpA* and part of *atp B* from *S. pneumoniae*.^[8] This study was reportedly conducted at Pneumococcal Reference Laboratory, Madrid, Spain. They detected an open reading frame *ant* in 2 optochin sensitive viridians streptococci (*Streptococcus mitis* and *Streptococcus oralis*), which was not found in *S. pneumoniae*, confirmed by Southern blotting technique. Comparisons of the chromosomal framework of the *atp* operon regions in *S. pneumoniae* and 2 isolates of optochin-sensitive viridians streptococci (*S. mitis* and *S. oralis*) characterized in their study and the nucleotide sequence of the *atpC-atpA-atpB* region, have strongly suggested the recombinant origin of optochin-sensitive viridians streptococci.

There have been reports of emerging optochin-resistant pneumococci in a few countries.^[1-3] Resistant strains have point mutations in either a- or c-subunit of the H + ATPase, and demonstrate optochin MICs 4- to 30-fold higher than susceptible isolates.^[9] Atypical presentation of pneumococci which was identified by pneumolysin detection in a similar case report by Kearns *et al.*^[10] reinforces the diminishing reliability of optochin sensitivity to characterize pneumococci in clinical samples.

Conclusion

Diagnostic laboratories should watch out for atypical characterization of pneumococci in clinical isolates.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not

be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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