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Letter to Editor

Microbiology Section

Growth of *Streptococcus pneumoniae* on Macconkey Agar: Possibility of Impossible?

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Dear Editor

Streptococcus pneumoniae (*S. pneumoniae*) is Gram-positive lanceolate-shaped diplococci which is a significant human pathogenic bacterium causing pneumonia, meningitis and various other infections. It is a fastidious organism and grows well in 5% carbondioxide (CO_2) and media containing blood (blood agar, chocolate gar) as growth requires a source of catalase (e.g. blood) to neutralize the large amount of hydrogen peroxide (H_2O_2) produced by the bacteria [1]. Hence, it does not grow on MacConkey agar (MA) due to the absence of blood as well as due to the presence of high concentration of bile in the medium which is inhibitory to the growth of *S. pneumoniae*. However, to the best of our knowledge, we are reporting the first and unusual case of *S. pneumoniae* growth on MA.

We received cerebrospinal fluid from a 21-year-old male patient who was admitted with fever, headache, vomiting and clinically had signs of meningitis. Gram stain revealed few pus cells. Biochemical parameters showed decreased glucose, moderately increased protein. Culture revealed pure growth of tiny opaque lactose-fermenting colonies on MA and alpha-haemolytic colonies on blood agar (BA) and chocolate agar (CA). Colony smear from all the three plates showed lanceolate-shaped Gram positive cocci in pairs and short chains. Identification and antibiotic sensitivity testing was done by Vitek-2 compact (BioMerieux) using GPC identification card (GP 21 342) and Streptococcal AST card (AST-ST01) respectively. The organism was identified as *Streptococcus pneumoniae* which was sensitive to all the antibiotics tested except benzylpenicillin. However, the growth of the isolate on MA created a dilemma and hence the same was sent for identification by MALDI-TOF method. The later identified the isolate as *Streptococcus pneumoniae* with score value of 2.114.

Several studies have highlighted evolving clinical problems with *S. pneumoniae*. Increasing resistance to antimicrobial agents, and failure of traditional optochin(ethylhydrocupreine) sensitivity had been a growing concern [2]. Dias CA et al., reported the characteristics of four optochin-resistant (Opt(r)) *Streptococcus pneumoniae* isolates from Brazil. All four Opt(r) isolates presented mutations in the nucleotide sequence coding for the c subunit of F(0)F(1) ATPase [3].

Clinical isolate with intermediate sensitivity to penicillin was first reported in 1967. Since then, penicillin non-susceptible *S. pneumoniae* has been reported with increasing frequency in many countries.The resistance to penicillin arises from mosaic mutation of penicillin binding protein (PBP) genes due to interspecies recombination of homologous genes [4].

Growth of *S. pneumoniae* on MA may be a beginning of the changing trend of the organism's fastidious growth requirements. One of the possible explanations for the same may be some mutation causing alteration in hydrogen peroxide producing capacity of *S. pneumoniae* and hence bypassing the need for catalase in the media used.Some strains of *S. pneumoniae* with such mutations have been used in experimental studies to create an environment with low concentration of H_2O_2 [5,6]. Pericone et al., used loss-of-function mutations of spxB gene in P62 and P878 strains of *S. pneumoniae* to study the effect of low H_2O_2 on rate of spontaneous gene mutation. The lower spxB expression resulting in under-expression of pyruvate oxidase results in the lower production of hydrogen peroxide. A spxB-defective pneumococcal mutant produces opaque colony and opaque variants show increased virulence for non-nasopharyngeal infections [5]. In our case also the colony morphology was opaque on MA, and the infection site was meninges. So, these findings provides an assumption that the spx-B mutation can be occurring in the clinical isolates like in our case thus leading to diminished production of hydrogen peroxide and hence not requiring catalase in the growth medium.

Due to unavailability of molecular methods at our institution the genetic analysis could not be performed in this case. However, it is advisable that in future if any such scenario is encountered by any institution the genetic analysis could be of great help.

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