

Rapid Detection of Klebsiella Pneumoniae by Capsular Polysaccharide Antigen

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Abstract—The aim of this study is to develop simple and rapid diagnostic method which can be utilized routinely for detection of Klebsiella pneumoniae. This study was done in India at DRDE, Gwalior. We had collected standard K.pneumoniae strain 3296 from Yamaguchi University, Japan and total fifty-nine clinical isolates of Klebsiella species from Armed Force Medical College, Pune, and Patel chest hospital, New Delhi. Bacteria were grown on trypticase soy broth and two Newzealand wistar white rabbit were immunized subcutaneously with purified capsular polysaccharide (CPS) of K.pneumoniae. Specificity of hyper immune sera raised against CPS was tested by counter current immunoelectrophoresis. We have generated four monoclonal antibodies, out of which two IgM producing clones (KP-1 & KP-2) and hyper immune sera against CPS antigen were used for agglutination test. KP-1, KP-2 and CPS hyperimmune sera showed different reactions. Out of the twenty biochemically confirmed K.pneumoniae clinical isolates, hyperimmune sera to CPS detected sixteen as positive whereas KP-1 reacted to eleven and KP-2 to thirteen of these isolates. One clinical Klebsiella species biochemically negative for K.pneumoniae and other Enterobacteriaceae organisms were negative by agglutination test to all the three antibody reagents. K.aerogenes, however, reacted to KP-2 and CPS hyperimmune sera. Standard K.pneumoniae was positive to all the three antibodies. The rapidity and ease of performance merited the hyper immune sera to CPS and IgM monoclonal antibody based agglutination test as the preliminary identification system for K.pneumoniae.

Keywords—Klebsiella pneumoniae; monoclonal antibodies; CPS; diagnostic method.

I. INTRODUCTION

Klebsiella is an important opportunistic pathogen, which was reported world wide. It can causes infections of respiratory tract, nasal mucosa, pharynx and generally results in primary pneumonia. Pneumonia is the most frequent nosocomial infection (30 to 33% of cases) among combined medical-surgical intensive care units participating in the National Nosocomial Infections Surveillance System [1]. Klebsiella species were found to be the most frequently isolated gram negative bacteria in cases of primary bacteremia [2]. Capsule plays a very important role in virulence so we have started our study with the aim to utilize capsular polysaccharide as an antigen to develop simple and rapid diagnostic method which can be utilized anywhere

without the use of any sophisticated equipments specially for field use as a preliminary routine test for detection of Klebsiella pneumoniae. The diagnosis of Klebsiella infection is made on the basis of clinical examination, isolation and identification of the organism. Laboratory identification is usually carried out by cultures, gram staining and a battery of biochemical tests. Bacterial isolation takes 24 hours and biochemical tests have their own limitations in that the results may be available only after 48 hours. Even then accurate identification at species level of the genus may not be possible in all cases. In clinical situations, the severity of infections does not warrant such a long period of investigation for an appropriate treatment to be started. Speed of diagnosis, therefore, is essential in the management of serious patients.

Hence, the need arises for reliable and quick serological methods for rapid diagnosis of Klebsiella infections. Several serological assays for detection of antibody and antigen have been developed and these include capsular swelling reaction, ELISA and so on. The problems with most of the reported antibody and antigen detection assays so far have been the low reproducibility, sensitivity and specificity with high cost. The reason being ,most of the antigenic moieties and polyclonal antibodies used are common to many related and unrelated bacterial species. As a result most of these assays have exhibited low potential in endemic areas due to ubiquitous nature of Klebsiella and shows extensive cross reactions among themselves.

Since there is a need to develop more sensitive and specific assay with monoclonal antibodies and capsular polysaccharide antisera. With this aspect in view, the present study was initiated with the following aims and objectives:

- 1) To develop hyper immune sera against capsular polysaccharide antigen of K.pneumoniae.
- 2) To develop rapid and simple antigen detection immunoassay with capsular polysaccharide antisera for the diagnosis of K. pneumoniae.
- 3) To compare the effectiveness of monoclonal and capsular polysaccharide polyclonal antibodies against K.pneumoniae by using agglutination method for detection of organism.

II. MATERIAL AND METHODS

We have collected standard and clinical isolates of *K.pneumoniae* from different places and utilized them for our study.

A. Bacterial Strains

1) *Standard cultures*: *K.pneumoniae* strain 3296, 01K1 & 01K2 and K-2 was obtained from Japan, New York and India respectively.

2) *Clinical isolates*: Fifty - four clinical isolates of *Klebsiella* species recovered from pus, urine and sputum samples (Armed Force Medical College, Pune, India), four from pneumonic patients and one from urinary tract infection (Patel Chest Hospital, New Delhi, India).

3) *Other bacteria*: *K. aerogenes*, *E. aerogenes*, *C. koseri*, *E. cloacae*, *Y. enterocolitica*, *Y. pseudotuberculosis* were obtained from microbiology division of DRDE, Gwalior, India.

B. Biochemical Identification of Bacteria

All the clinical isolates were examined morphologically for colony characterization on agar media. Those exhibiting mucoid colonies were processed for biochemical testing. Biochemical tests employed were urease production, citrate utilization and fermentation of sugars. Sugar fermentation tests performed were sucrose, glucose, mannitol, lactose, adonitol, dulcitol, melibiose and esculin. Indole test and H₂S production on TSI agar, oxidase, catalase and nitrate were also carried out. Beside these tests, motility and growth of organism in KCN were also checked. For biochemical tests standard procedures were used [3].

C. Purification of Capsular Polysaccharide

Bacteria were grown on trypticase soy broth at 37°C with shaking at 100 rpm in a shaker incubator for 16 hours. Organisms were harvested by centrifugation at 10,000 Xg for 30 minutes. Cetavlon, was added to a final concentration of 0.5 %. After stirring for 30 minutes at room temperature, the precipitate was collected by centrifugation. The material was dissolved in 1M CaCl₂ solution and 25% ethanol was added to it in equal amount. After stirring for 60 minutes at room temperature, the precipitated nucleic acid were removed by centrifugation at 10,000 X g for 30 minutes. The CPS was precipitated from the resultant supernatant by addition of equal volume of 80% ethanol. The CPS was dissolved in distilled water and extracted three times with an equal volume of chloroform - butanol solution (5:1). The pooled water phases were extensively dialyzed against distilled water at 40°C and then vacuum concentrated. Finally the solution was centrifuged at 100,000 X g for 16 hours for the removal of lipopolysaccharide. The resulting pellet was discarded and supernatant containing CPS was collected. CPS was purified [4] followed with sugar estimation [5].

D. Immunization with Capsular Polysaccharide Antigen

Rabbit was immunized with 800 ug / dose of purified CPS of *K.pneumoniae*. Antigen was given subcutaneously with adjuvant. Five doses of same amount were given at

seven days interval each with adjuvant. Antibody titer was checked by counter current immunoelectrophoresis.

E. Agglutination

Bacterial suspension of all clinical isolates of *K.pneumoniae* along with negative and positive control were made in glycine saline buffer and a homogeneous suspension was prepared. Equal volume of bacterial suspension and IgM monoclonal antibodies KP-1, KP-2 and antibodies to capsular polysaccharide were added on slides separately. They were mixed properly with stick and observed for agglutination reaction within five minutes.

III. RESULTS AND DISCUSSION

The standards and clinical isolates of *Klebsiella* species showed morphological characteristic bacterial colonies on BHI and nutrient agar exhibiting mucoid colonies of viscidity consistency on these agars. Capsule production was seen by cultivating the organism on bromo thymol blue lactose agar. All the four standard *K.pneumoniae* (3296, 01K1, 01K2 and K2) strains produced navy blue colored colonies indicating capsule production. The biochemical characterization of standards and clinical isolates of *Klebsiella* were examined by battery of biochemical tests. Standard *K.pneumoniae* cultures showed positive reaction for urease production, citrate utilization, catalase reaction and fermentation of sugar like glucose, lactose sucrose, mannitol, adonitol, melibiose and esculin. Organism showed negative test for indole production. There was no H₂S production on Triple Sugar Iron agar but growth of organism was seen in KCN.

All the fifty-nine clinical isolates exhibiting colonies similar to *Klebsiella* species were tested biochemically. Thirty – six clinical isolates showed typical common biochemical reaction pattern similar to the one seen with *Klebsiella* species, being positive to glucose, lactose, sucrose, mannitol and negative to oxidase and indole. When tested by second battery of biochemical reactions that included some rare sugars (adonitol, melibiose, esculin and dulcitol), twenty of them exhibited reactions attributable to majority of *K.pneumoniae* sub species *pneumoniae* strains, being positive to adonitol, melibiose, esculin, urease, and citrate. They also showed growth in KCN. The rest of the isolates had variable reactions with these tests.

We have generated and collected KP1, KP2 (IgM) monoclonal antibodies and CPS polyclonal antibodies which were used for agglutination test. These antibodies showed different reactions against *K. pneumoniae*. Out of fifty - nine clinical isolates morphologically resembling *Klebsiella*, twenty- one clinical isolates were found positive for *K. pneumoniae* at biochemical and SDS-PAGE characterization. Out of these twenty – one clinical isolates, hyper immune sera to CPS detected 76% samples as positive and unable to react 24% samples of *K. pneumoniae* whereas KP1 reacted to 52% clinical isolates and showed no reaction to 48% samples. KP2 detected 62% of these isolates as positive and 38% were detected as negative samples. We have utilized *K. aerogenes*, *E. aerogenes*, *C. koseri*, *E. cloacae*, *Y. enterocolitica*, *Y. pseudotuberculosis* as negative control and found that KP1 is highly specific with low sensitivity as it

does not showed any cross-reactivity for negative controls but sensitivity of this clone is only 52% however, KP2 and CPS antisera showed cross - reactivity against *K.aerogenes* but not against any other negative control. CPS antisera showed the highest sensitivity out of these three antibodies as it has detected sixteen as positive and five as negative. Standard *K.pneumoniae* was positive to all the three antibodies. The details of data is shown in figure A.

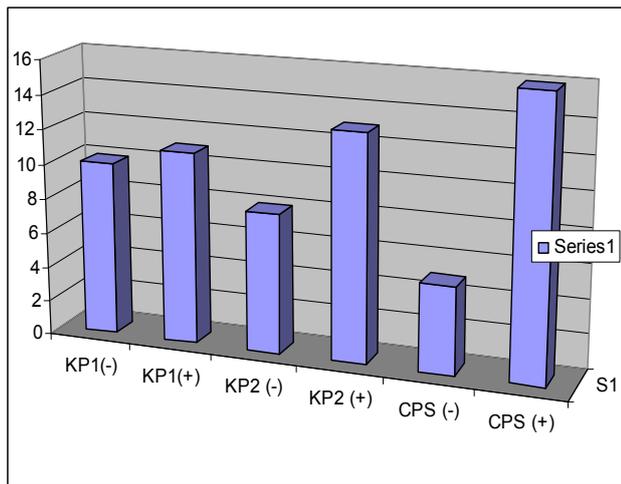


Figure A : Detection of *K.pneumoniae* clinical isolates by agglutination test

IV. CONCLUSION

IgM producing clones KP1, KP2 and CPS antibodies were utilized for the agglutination test system. Out of these three antibodies, We found that CPS antibodies showed the highest sensitivity 76% for clinical isolates. The rapidity and ease of performance merited the HIS to CPS polyclonal antibody based agglutination tests as the preliminary identification systems for *K.pneumoniae*. This newly developed CPS polyclonal antibody based agglutination test is simple and quick, which can possibly replace the time and resources consuming conventional biochemical identification in routine bacteriological laboratories. The test can also be utilized in peripheral disease surveillance / clinical laboratories for rapid identification of *K. pneumoniae*. Direct detection of antigens from clinical samples needs to be explored with the specific antibodies as reported here. For future research, we can generate monoclonal antibodies against purified CPS antibodies and utilized that for simple and more reliable preliminary testing purposes, specially in fields for soldiers and villagers where they don't have advanced equipments for testing.

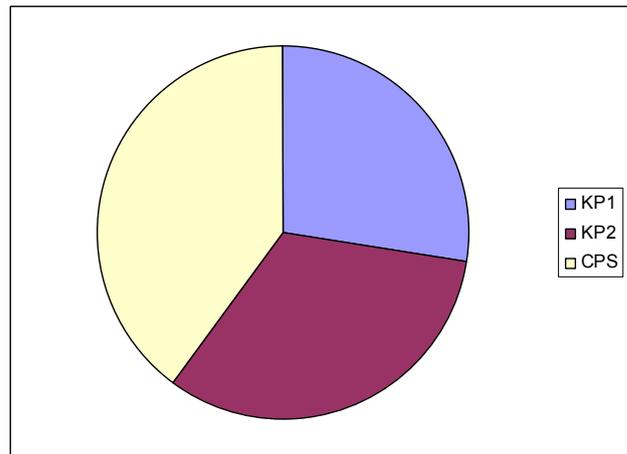


Figure B: Comparative analysis of newly developed detection system for specificity and sensitivity of monoclonal KP1, KP2 and CPS polyclonal antibodies against *K.pneumoniae*

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