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Detection of metallo β-lactamase producing *Klebsiella pneumoniae* in a neonatal septicemia

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ABSTRACT

A 10-day old preterm neonate, appropriate for the date, was admitted for lethargy and feeding intolerance. By culturing its blood, the antibiogram of the causative bacterium was ascertained by both Kirby-Bauer disc diffusion method and the Vitek2 system. Due clinical steps were taken for survival of the baby. The baby was suffering from septicemia. The causative bacterium was *Klebsiella pneumoniae* (*K. pneumoniae*). The isolated strain was found resistant to a total of 17 antibiotics; the strain was positive for extended spectrum β-lactamase production and resistant to two carbapenems, imipenem and meropenem. The baby could not survive. The baby was infected with an appalling strain of *K. pneumoniae* with a capacity to produce metallo β-lactamase overriding carbapenems, which is found to present in the state of Odisha, India.

1. Introduction

Multidrug resistance has been an emerging problem clinically, especially for aged and immune compromised patients, and additionally neonates with undeveloped body physiology have the least capacity against infection from multidrug resistant (MDR) pathogens. The production of extended spectrum β-lactamase (ESBL) is one of the important mechanisms of drug resistance and the emergence of CTX M β-lactamase by Enterobacteriaceae has been a matter of clinical concern[1,2]. In due course, strains of Klebsiella pneumoniae (*K. pneumoniae*) have emerged with an armamentarium of modifications of outer membrane proteins playing crucial role in antibiotic-efflux mechanisms, generating uncontrollable *K. pneumoniae* strains[3]. A Swedish national was hospitalized in New Delhi with infections with *K. pneumoniae* and *Escherichia coli* (*E. coli*), and both strains had the plasmid, blaNDM-1, which was easily transmitted nosocomially[4]. The enzyme, 'New Delhi β-lactamase' (NDM-1) was detected biologically and epidemiologically in patients of the Indian sub continent[4].The new, repugnant blaNDM-1 gene in the strain of *K. pneumoniae* was with the carbapenamase, consequently resistance to imipenem and meropenem (carbapenems) is offered[5]. The infection with *K. pneumoniae* could be a graver problem, because it causes bacteremia, pneumonia and septicemia, alike in all age groups[6], reported from Germany, for example. The underlying mechanism of carbapenem resistance in ESBL positive *K. pneumoniae* isolates is the absence of porin channels (Omp K35 or Omp K36) for antibiotic uptake[7], combined with AmpC β-lactamases for expression of carbapenemase[8].

This report records detection of an infamous strain of *K. pneumoniae*, which has resistance to many commonly used drugs, including the rarely used imipenem (IMP). Thus, this strain is labeled as metallo β-lactamase. By the by, the strain was monitored through Vitek 2 system for

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confirmation as a MDR pathogen.

2. Case report

The second part of a twin, a seven-day old preterm (gestational age, GA, 31 weeks) neonate with a low birth weight, 1.25 kg, was admitted to our neonatal intensive care unit, from a tertiary care hospital for lethargy and feeding intolerance. It was ascertained that the neonate was neither small for GA nor for date. It was reported that it had a normal vaginal delivery in that hospital without any perinatal asphyxia, but there was a history of premature rupture of membrane (PROM) of around 24 h. Antibiotics (cefotaxime and netromycin, for 7 d) and caffeine citrate were given to the baby, and gavage feeding was followed for further management. On admission, the baby was lethargic, normothermic, euglycemic, haemodynamically stable, but was with icterus up to palm and sole. Antibiotics (piperacillin/tazobactam and gentamicin) were given empirically. Further, the baby deteriorated with intermittent tonic seizure, apnoea and hypoglycemia. Therefore, anti-convulsants (lorazepam and phenobarbitone), ionotropes, injection vancomycin, and immunoglobulin were given. Further, the baby became haemodynamically progressively unstable, and eventually it was ventilated in synchronized intermittent mandatory ventilation, with pressurized oxygen. However, the expected pizzazz was lacking, it was slumbering, the rapid deterioration was evident leading to an impasse, and death occurred in spite of conflated supports. But, the first part of the twin is healthy.

Blood counts, electrolytes, bloodgas (ABG) reports and chest X-ray picture were normal. There were a high billirubin content (total 18 mg/dL, direct 1.8 mg/dL), without any sign of kernicterus, high micro-'erythrocyte sedimentation rate' and C-reactive protein (more than 7.2 mg/dL). From the blood culture, *K. pneumoniae* was identified, with the help of conventional biochemical methods, as per clinical and laboratory standards institute (CLSI) guidelines[9]. Moreover, the isolate was identified, in parallel by the Vitek 2 system (Biomeriux Vitek system). Antibiotic (HiMedia)-susceptibility tests of the isolate were performed by the disc-diffusion method according to CLSI guidelines, as done before[10], also by the Vitek 2 advanced expert system, by which values of minimum inhibitory concentration for all antibiotics were determined (Table 1). Phenotypical detection of ESBL by double disc synergy test (DDST) method was done, as described elsewhere[10]. For the phenotypical detection of metallo-β-lactamase, the imipenem-EDTA-DDST was done, according to CLSI guidelines: the organism was inoculated on to plates with Muller-Hinton agar for a lawn culture; an disc of imipenem (10 μg/disc) was placed at a distance of 20 mm centre to centre from a blank disc containing 10 μL of 0.5 M EDTA. Enhancement of the zone of inhibition in the area between the imipenem disc and the EDTA disc, in comparison with the zone of inhibition on the far side of the disc with imipenem was interpreted as the positive result. The *K. pneumoniae* strain was found resistant to 17 antibiotics, ESBL-positive and resistant to imipenem and meropenem (Table 1).

Table 1
Antibiogram of the isolated *K. pneumoniae* strain.

Antibiotics	MIC (μg/mL)	Status
Ampicillin/sulbactam	≥32	R
Ticarcillin	≥128	R
Piperacillin	≥128	R
Piperacillin/tazobactam	≥128	R
Ceftazidime	≥64	R
Ceftriaxone	≥64	R
Cefoperazone/sulbactam	≥128	R
Cefepime	≥64	R
Imipenem	≥16	R
Meropenem	≥16	R
Amikacin	≥64	R
Gentamicin	≥16	R
Tobramycin	>16	R
Ciprofloxacin	≥4	R
Levofloxacin	≥8	R
Tetracycline	≥16	R
Trimethoprime/sulfamethoxazole	≥320	R

Note: R=resistant.

3. Discussion

Viewed from the trenches of public health, carbapenem resistance in an ESBL-positive Gram-negative enteropathogenic bacterium infecting neonates is the most serious threat from MDR pathogens, ever seen. This is the first report from our hospital on carbapenem resistance *K. pneumoniae*. In our hospital, 33.33% ESBL strains of *P. aeruginosa* were reported recently [10]. Virulent MDR enteropathogens (*Klebsiella, Salmonella, Pseudomonas, Shigella, Enterococcus, Vibrio* and a few more) are active in the non-hygienic poorer communities of developing countries; and these are the causative organisms of high infant mortality and out-break of infrequent fervent episodes[11]. Today, three Gram-negative bacteria resistant to all the major classes of antibiotics have been emerged and these could be cited as ferocious pandrug resistant (PDR) bacteria; more prominently, *Pseudomonas aeruginosa, Acinetobacter baumannii* and *K. pneumoniae* cause utmost comorbidities and frequent immature mortality of all age groups[12]. Of course, the concept of PDR is not yet consensus in literature, despite its clear etymological meanings, 'resistant to almost all commercially available antimicrobials', 'resistant to all antimicrobials routinely tested' and 'resistant to all antibiotic classes available for empirical treatment'. Moreover, the expression of carbapenemase fortified *K. pneumoniae* as a pathogen and saving a neonate from

this PDR strain was abominably intractable, rather was exasperating in clinical management.

The present case, unlike most other cases of under-5 mortality due to under-nutrition or diarrhoea or pneumonia or malaria, is a special case of septicemia by a strain of metallo-β-lactamase *K. pneumoniae*, prevalent in eastern India, which was promoted to the neonate probably due to PROM. As known, globally mortality rate of neonates has decreased in the last decade, from 12 million in 1990 to 6.9 million in 2011, recorded in a WHO estimate[13,14], and this country has 24% of under-5 death, and India is one among 5 leading countries of under-5 mortality. Levels and trends of under-5 mortality and survival of infants as well as neonate have been decreasing worldwide, but in several developing countries the levels are still higher[15-17]. But, infant mortality rates, 35% in Sub-Saharan African, 45% in Eastern and Southern Africa, 28% in West and Central Africa, 48% in Middle East and North Africa, 43% in South Asia, 59% in East Asia and Pacific, 61% in Latin America and Caribbean, 56% in Central Eastern Europe/Commonwealth Independent States were recorded, whereas the world figure was 39%[18].

Conflict of interest

The authors declare they have no conflict of interests.

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