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Outbreak investigation of NDM-producing *Burkholderia cepacia* causing neonatal sepsis in Pakistan

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Plain language summary

Neonatal septicemia (NS), or blood poisoning, is a dangerous illness in newborns. It is caused by bacteria or other infections entering the blood and spreading. Pregnancy, labor, delivery and exposure after birth can result in infection of the newborn. NS kills 700,000 babies worldwide, mostly in low- and middle-income countries. Burkholderia cepacia complex (BCC) bacteria can cause infections in people with weaker immune systems or other disorders. They are particularly dangerous in hospitals as they can cause chronic lung problems. This study collected blood samples from newborns with blood poisoning. Most samples that contained BCC were not

susceptible to drugs. Four of the newborns carried the same bacteria, indicating that hospital staff should practice hand washing and equipment and environmental cleaning to prevent the spread of the bacteria.

Abstract

Aim: To investigate the outbreak of *Burkholderia cepacia complex* (BCC), mortality, antimicrobial resistance, and associated risk factors in the NICU. **Method:** 18 blood culture samples from neonates and 20 swabs samples from different NICU surfaces were collected. The VITEK 2 was used to confirm the isolates and generate the antibiogram. PCR was used to identify *bla*NDM. **Results:** 18 samples tested positive for BCC, and 10/18 (55.5%) of the neonates died.. 13/18 (72%) of the neonates had LONS, and10 (55%) had low birth weight, Resistance to minocycline and chloramphenicol was 100%, and 72.2% to meropenem. . 72.2% NDM gene was found in neonates and was 20% from the environment. **Conclusion:** I Outbreak of NDM-producing BCC resulting in high neonatal mortality.

Keywords: Neonatal septicemia, NDM, antimicrobial resistance (AMR), Burkholderia cepacia complex (BCC)

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Introduction:

An estimated 2.5 million newborns died within the first 28 days of life globally, due to infections particularly sepsis, meningitis, and pneumonia with low-middle-income countries accounting for nearly 80% of these deaths [1]. The risk of hospital-acquired infection (HAI) is incredibly high for

neonates admitted to hospitals in low-resource settings, and it is associated with overcrowding and understaffing, as well as lax infection control protocols [2]. Sepsis is defined as a potentially fatal organ dysfunction syndrome caused by an abnormal host response to infections. When an infection you already have sets off a series of events throughout your body, it results in sepsis. It is causing infections typically begin in the gastrointestinal system, urinary tract, skin, or lungs. Sepsis can quickly cause tissue damage, organ failure, and death if not treated promptly [3]. According to the World Health Organization (WHO), septicemia accounts for 20% of all global deaths, with children ≤5 accounting for half of all deaths [4]. Neonatal sepsis (NS) is divided into two types: early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS). EONS is 0-7 days of life and mostly acquired vertical infection (from mother to fetus) while LONS is 8-28days and linked with mainly horizontal transmission or HAI. *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, Group B Streptococcus* and *Burkholderia cepacia complex* (BCC) are the most notorious pathogens responsible for HAI and outbreaks mainly in the neonatal intensive care units (NICU) [5, 6]. In honour of its discoverer, BCC, formerly known as *Pseudomonas cepacia*, was given its genus in 1992. Presently, BCC includes seventeen closely related species (formerly known as genomovars). BCC are the aerobic, catalase-producing, glucose-non-fermenting Gram-negative bacilli present in soil and damp habitats that can survive and grow in nutrient-depleted water. BCC cause serious illness in critically ill and immune-compromised patients and is not considered part of normal human flora. It mainly affects immunocompromised children especially those who have a history of malignancy, congenital heart disease and prematurity. [7]. Hospital outbreaks have been reported because intravenous fluids, nebulizer solutions, contaminated disinfectants, medical devices, and catheters are potential reservoirs for BCC [8]. Their infections are proving difficult

to treat because of intrinsic resistance to many antibiotics, including β-lactams, aminoglycosides, antipseudomonal drugs, and polymyxins and pose therapeutic challenges [9]. Resistance to β-lactam antibiotics mediated by reduced permeability and the production of plasmid-mediated class C/AmpC lactamases, extended-spectrum-β-lactamases (ESBL), carbapenemase, and notably Metallo-β-lactamases (MBLs) [10]. MBLs are broadly classified into two categories; Ambler classification based on genetics and Bush Jacoby classification based on chemical nature. Ambler classification is further divided into A-D and Bush Jacoby classification is divided into 1-4. New Delhi Metallo-β-lactamase (NDM) refers to the enzymes of the MBLs belonging to the class B1 superfamily that hydrolyze carbapenems; considered the last resort to treat infections produced by multidrug-resistant pathogens [11]. The NDM gene was discovered in *E. coli*, and *K. pneumoniae* urine isolates recovered from a Swedish patient in New Delhi, India [12]. NDM-producing bacteria are not only resistant to β-lactam drugs but also to other classes of antibiotics including aminoglycosides, quinolones, and fluoroquinolones. NDM is mostly reported in the *Enterobacterales* now being reported in non-fermenters including BCC in many countries [13]. At the national level, there is less or no data available on the outbreak produced by the NDM-producing BCC in NICU in a tertiary care hospital. As a result, this study aims to investigate an outbreak of NDM-producing BCC in NS and associated risk factors in the NICU, as well as the phenotypic and genotypic determination of the NDM with a focus on β-lactamase resistance.

MATERIALS AND METHODS

Ethical consideration and clinical history of the patients

This cross-sectional study was conducted from February to May 2021 at the Department of Pathology, Fatima Memorial Hospital (FMH) Lahore, with ethical approval from the Institutional Review Board (FMH-01-2021-IRB-865-M). Further, informed written consent for blood sample collection obtained from the parents or guardians of the neonates was obtained. Ethical approval from each neonatal parent or guardian was obtained following the Declaration of Helsinki from the Institutional Review Board, Fatima Memorial Hospital Lahore. The neonate in this study was classified as being between 1 and 29 days old. Blood samples from neonates with clinical suspicion of septicemia were collected from the NICU ward by the trained nursing staff and sent to FMH's Microbiology laboratory for culture and sensitivity testing. The suspected neonates suffering from heart rate >90 beats/mints, respiratory rate >20 breaths/mints, fever (>38ºC) and hypothermia (<38 ºC), leukocytosis, and leukopenia were included in the study. The hospital is a tertiary care facility with 500 beds. The infection spread in the NICU and as a potential risk factor, the medical records of all neonates suspected of septicemia were reviewed, including age, gender, date of hospital admission, date of discharge, hospital stay, predisposing factors, gestational age, indoor and outdoor temperature, pulse rate, heartbeat, weight, and patient outcome. Additionally, haematological factors such as white blood cells, C-reactive protein, and platelets were taken into account. A surveillance sampling was conducted by collecting the swabs from different surfaces of the NICU from a tertiary care hospital in Lahore.

Neonatal blood collection and the surveillance NICU ward

1-3ml blood was collected by trained nurses from clinically suspected neonates in Bactec/ALERT PF blood culture bottles (Biomerieux, France) to confirm the laboratory diagnosis of sepsis. For up to five days, the bottles were incubated and monitored using the Bactec/ ALERT system (Biomerieux, France). The key concept of the Bactec/ALERT is that when microorganisms are

present in culture vials that cause them to metabolise the nutrients in the culture medium, they metabolise the nutrients and release carbon dioxide into the medium. A dye in the sensor at the bottom of the vial reacts to carbon dioxide. This alters the amount of light that the fluorescent material in the sensor can absorb. All positive blood culture bottles were subcultured on 5-10% sheep blood agar and MacConkey agar, and plates were incubated at 37 °C overnight for 24 hours aerobically.

For NICU surveillance, 20 Amies transport swabs samples were collected from different areas of the NICU, including incubator humidifier (n=3), tap water (n=2), water sink (n=2), incubator surface (n=2), suction bottle (n=4), respiratory devices (n=4), and the suction catheter (n=3). Swabs samples were inoculated on the blood and MacConkey agar plates, and incubated for 24 hours at 37 °C aerobically.

Initially, the isolates were identified based on colony morphology, culture characteristics and Gram stain and further confirmation was carried out by the VITEK 2® system (bioMérieux SA, Marcy l'Etoile, France) confirmed both clinical and environmental bacteria using GN cards by utilizing 64 different biochemical substrates. The VITEK 2 systems employ advanced colorimetry™, an identifying technology that enables routine clinical isolate identification. Advanced colourimetry includes the following features, high interspecies discrimination. Multiple choice and misidentified species are uncommon.

Testing for antimicrobial susceptibility

Antimicrobial resistance is a major cause of clinical insensitivity to treatment and rapid progression to sepsis and septic shock. The antimicrobial susceptibility of *B. cepacia* isolates was

determined using the Kirby-Bauer disc diffusion method and for the minimum inhibitory concentration (MIC) by the VITEK 2 system, under Clinical and Laboratory Standard Institute (CLSI) guidelines [14]. The VITEK® 2 antimicrobial susceptibility testing (AST) cards work based on the broth microdilution minimum inhibitory concentration approach. The VITEK® 2 AST card is essentially a miniaturised, shortened, and automated version of the doubling dilution process for microdilution-determined MIC. In short, 0.5Mcfarland bacterial suspension was made and placed inside the VITEK 2 GN cards. The cards were placed inside the VITEK 2 system for bacterial identification. The following antibiotics were used for the antibiogram such as ceftazidime, co-trimoxazole, levofloxacin, minocycline, imipenem, and meropenem. The interpretation of the antibiotics was determined as per CLSI guidelines.

Phenotypic detection of carbapenemase enzyme

Carbapenemase was detected using the Modified Hodge's test (MHT), as previously described [15]. First, 0.5McFarland of ATCC 25922 *E. coli* control strain was prepared in sterile normal saline. The isolates were lawned on the Mueller Hinton Agar (MHA) plate using a sterile swab. A meropenem disc (10µg) was placed in the centre of the MHA plate and test organism, negative control and positive control were streaked from the edge of the disc to the edge of the plate. The MHA plate was incubated at 37 ºC for 18-20 hrs in the aerobic incubator. After incubation, if bacteria showed the clover leaf-like indentation the MHT was considered positive as described in the CLSI guidelines.

Phenotypic detection of Metallo-β-lactamases (MBL)

The double disc synergy method was used to detect MBL as described previously [15]. In short, MBLs require Zn^{+2} as a cofactor for their enzymatic activity. By treating them with a chelating

agent such as ethylene diamine tetraacetic acid (EDTA), their functional activity can be lost. On the MHA plate, 0.5McFraland bacterial suspension was lawned. Two meropenem (10µg) and two ertapenem (10µg) discs were placed, and one meropenem and ertapenem disc was treated with 0.5M EDTA solution. Bacteria were MBL producers if the EDTA discs had a zone of inhibition greater than 5mm when compared to non-EDTA discs.

Molecular identification of *bla*NDM-1

After phenotypic confirmation, the bacterial DNA of the isolates was extracted using a commercially available DNA extraction kit (TIANamp, China). The bla_{NDM} was identified using gene-specific primers which were commercially synthesized (Macrogen, Korea). The primers were, NDM-F 5′ATGGAATTGCCCAATATTATGCAC-3′ and NDM-R 5′-TCAGCGCAGCTTGTCGGC-3′ These are the following conditions applied for the amplification of the NDM gene; initial temperature: 95°C for 60sec, secondary temperature: 95°C for 45 sec, annealing: 58°C for 45 sec, primary extension: 72°C for 60sec and final extension: 72°C for 5min and ∞ at 4°C.After the amplification, 1.5% agarose gel was prepared in 1X TAE buffer. The amplified DNA product (10µl) was loaded in each well along with a DNA ladder (100bp) and run the electrophoresis (Bio-Rad, UK) at 100V for 30 minutes. The gel was placed in the GelDoc system (Bio-Rad, UK) to visualise the NDM bands (621bp) under UV light. The band was compared with the DNA ladder.

DNA sequencing

Further, the amplicon DNA was shipped to Euroffin UK for Sanger-based DNA sequencing. 15µl of purified DNA with the concentration of 10ng/µl was added into the sterile Eppendorf with 2µl of NDM forward and NDM reverse primers with a concentration of 10µM. The DNA sequence

data was obtained and analysed by different bioinformatics tools. First, the DNA sequence was analysed by the Genious software (http://www.geneious.com/). Further, the data was analysed by sequence similarity and region locus similarity using National Center for Biotechnology Information (NCBI) database and Basic Locus Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/). The aligned sequences were compared with other NDM variants sequences in the NCBI database.

RESULTS

Patient's clinical characteristics

The *Burkholderia cepacia complex* was found in all 18 neonatal blood cultures in the NICU of the tertiary care hospital. The majority of these isolates were recovered from males (11; 61%) rather than females (7; 39%), with a male-to-female ratio of 1.5:1 The ages ranged from 1 to 29 days, with a mean of 6.94 days. Ten (55%) of the neonates were delivered via caesarean section, with the majority of these being born in the hospital (11; 61%). 13 (72%) of the neonates had LONS, 5 (28%) EONS, 10 (55%) low birth weight (≤2kg), and 15 (83%) had a hospital stay of ≥72 hours. With septicemia, 14 (78%) of the neonates have hyperthermia, 10 (55%) have tachypnea and lethargy, and 16 (89%) have poor feeding. Furthermore, haematological findings revealed that 15 (83%) have high C-reactive protein (>6mg/dl), 14 (78%) have high white blood cell count (>10,000/cmm), and 8 (45%) have low platelet counts (50,000/cmm) (Table 1).

Characteristic of neonatal septicemia

In the NICU of the tertiary care hospital, BCC were recovered from all neonatal septicemia. The most concerning finding was the deaths of 10/18 (55.5%) neonates who had BCC infection. Among these, 4/10 (40%) neonates died with preterm, 2/10 (20%) with syndromic and 2/10 (20%)

with intrauterine growth retardation (IUGR). The age ranges from 1-29 days with a mean age was 6.94 days on average. 8 (44.4%) patients stayed in the NICU for one to seven days, 7 (38.8%) for seven to fourteen days, and 3 (16.6%) for more than fourteen days. In addition, 2 (11.1%) patients have the syndrome, followed by 3 (16.6%) patients with IUGR, and 8 (44.4%) patients with preterm birth. Further, 12 (66.6%) had EONS ranging from 1 to 3 days of life and 6 (33.3%) had LONS with an age range from 9 to 29 days of life (Table 2).

Phenotypic confirmation of carbapenemase and MBL production

The phenotypic test was employed for the validation of carbapenemase and MBL enzymes. Phenotypic The studies of the carbapenemase and MBL revealed that 13/18 (72.2%) BCC produced carbapenemase using the MHT, and 12/18 (66.6%) produced MBL using the double disc synergy method (Figure 1). The BCC isolates were subsequently examined for the molecular confirmation of blaNDM.

Profile of antimicrobial resistance

The Kirby-Bauer disc diffusion method was used to perform the preliminary screening of the antimicrobial susceptibility profile, and the VITEK 2 system was used to perform the subsequent confirmation. The AST results displayed that all of the BCC isolates were found to be resistant to minocycline (≥32µg/ml) and chloramphenicol (≥32µg/ml), followed by 14 (77.7%) to levofloxacin (≥8 µg/ml) and 13 (72.2%) to ceftazidime (≥32µg/ml), imipenem (≥16 µg/ml), and meropenem (≥16 µg/ml). According to the WHO's classification of antibiotics (AWaRe), there were two antibiotics that belonged to the "Access" group (co-trimoxazole and chloramphenicol), four antibiotics that belonged to the "Watch" group (ceftazidime, levofloxacin, imipenem, and meropenem), and one antibiotic that belonged to the "Reserve" group (minocycline).

Molecular detection of *bla*$_{NDM}$ carried by BCC isolates

According to the molecular identification of blaNDM, the results of the molecular profile showed that 13/18 (46.4%) carbapenemase-producing BCC tested positive for the blaNDM. In addition, 7 out of 18 neonates who were infected with the NDM-producing BCC died. This represents a mortality rate of 38.8%. (Figure 2 and Table 3).

Antimicrobial susceptibility profile of NICU environment samples

NICU wards had a total of twenty environmental swabs taken from various areas, such as suction bottles (n=4), respiratory equipment (n=4), incubators (n=3), the suction catheter (n=3), tap water (n=2), the sink drain (n=2), and the surface of the incubator (n=2). Positive results were found in 4/20 (20%) of the samples. Each of the following contributed one BCC to the collection: the incubator, the water sink, the suction bottle, and the suction catheter. The environmental BCC isolates were resistant to all the WHO AWaRe classes of antibiotics including ceftazidime (≥32 µg/ml), imipenem (≥16 µg/ml)., meropenem (≥16 µg/ml)., levofloxacin (≥8 µg/ml), minocycline (≥32µg/ml), and chloramphenicol (≥32µg/ml), according to the antimicrobial susceptibility profile.

Molecualr idebntifiaction of *bla*$_{NDM}$ containing BCC in envirnemntal samples

Amplification with PCR and Sanger sequencing analysis were the two methods that were used to determine the NDM gene. In addition, there were 4/20 (20%) BCC samples that tested positive for the blaNDM. The NDM gene was recovered from an incubator, a sink drain, a suction bottle, and a suction catheter in each of the four samples (Table 4).

Infection prevention strategies in the NICU

Infection control measures including education of hospital health workers, severe infection control measures, education of healthcare workers, and decontamination of the NICU's medical equipment including ventilators, baby incubators, baby corts, suction tubes, ambo bags, and ventilators. Infection control methods include education of hospital health workers. Following the completion of a surveillance study of the NICU, the ward was fumigated and decontaminated. Additionally, training was provided on hand hygiene, cleaning, cleaning of the patient's surrounds, donning clean aprons while dispensing clean materials, and donning clean aprons while preparing clean materials for the production of new patient beds. Other training topics included the correct donning and doffing of gloves, the correct disposal of bio waste, and training on personal protective equipment. During the staff work, the committee nurse participated in observations and provided criticism. In addition, education was offered to the personnel regarding the significance of BCC as well as BCC infections. Following the implementation of infection control measures, the NICU did not have any BCC strains in its samples.

DISCUSSION

Burkholderia cepacia complex is the notorious nosocomial pathogen prevalent in NICU that can be found in antiseptics, nebulizer solutions, ventilator tubing, and indwelling catheters. Invasive medical devices and the incorrect use of broad-spectrum antibiotics are usually linked to prolonged hospital stays for various medical or surgical operations. These stays are frequently caused by the patient requiring multiple interventions. An outbreak case was defined as a neonate who had a clinical suspicion of sepsis (fever, tachycardia, tachypnea, leukocytosis, or leukopenia, with or without hypotension) and one or more BCC-positive blood culture results. In the current investigation, all BCC were obtained from neonatal septicemia in the NICU. The majority of the

patients had LONS, had a low birth weight (less than 2 kilograms), and had spent less than 72 hours in the hospital. Two of the clinical signs that occurred in neonates were hyperthermia and poor eating. Septicemia was proven by the elevated level of C-reactive protein as well. These findings are in line with data that were only recently reported from an epidemic of BCC in Peshawar, Pakistan's neonatal intensive care unit. [16]. Another study from India documented 12 cases of neonates in the NICU with septicemia in a tertiary care hospital [17]. A Gambian study documented the sequential outbreak of BCC and MDR *K. pneumoniae* in the NICU of a tertiary care hospital [4]. Further, an outbreak of neonatal septicemia in the surgical unit was also reported in another study from India [18]. All of these factors, in addition to BCC's inherent ability to survive on moist surfaces within the environment, contribute to their effectiveness as infections in healthcare facilities. The most pressing problem with these organisms is that the virulence of sepsis is not significantly different from the virulence of other Gram-negative organisms, and given the length of our patients' stays in the hospital, the organism may become resistant to many antibiotics. The identical BCC strains were cultivated from every feasible source, including the incubator, the respirator, and the surfaces in the NICU ward. These are some of the potential factors that led to the BCC outbreak. Several studies [19-22] have found contamination of BCC in the hospital environment and during the manufacturing of different products and after the product has been opened. According to a recent outbreak report, the contaminated ultrasound probe gel used in ultrasound procedures resulted in *B. cepacia* bacteremia in patients [23]. Further, a Turkish study found the presence of BCC in mouthwash solutions in tertiary care hospitals [5]. Our isolates' minimum inhibitory concentration determination revealed multidrug resistance. In the present study, our investigation demonstrated resistance to ceftazidime while previous studies reported sensitivity to ceftazidime and other cephalosporins. The BCC recovered from patients and the

NICU environment displayed the same biological characteristics and antimicrobial-resistant profile. The previous data suggested that BCC are intrinsically resistant to various classes of antibiotics including aminoglycosides, quinolones, first, second and third-generation of cephalosporinsand left limited treatment options. The medicines useful against BCC were levofloxacin, meropenem, co-trimoxazole, ceftazidime, and minocycline as per CLSI guidelines. However, the combination of the antibiotic is considered effective for drug-resistant BCC [24, 25]. Worryingly, 10/18 (55.5%) neonates died as a result of BCC infection in this study. Previously studies reported neonatal mortality due to BCC infections globally. A Korean study reported 41% neonatal mortality [26], 25.9% in Singapore [27] and 50% mortality in Malaysia [28]. NDM are β-lactam genes that produce resistance to a variety of antibiotic classes, including carbapenems. We identified 13/18 (72.2%) NDM-producing BCC cases and 4/20 (20%) of the environmental samples were NDM producers. The NDM-producing BCC clinical isolates were documented in an Indian study [29]. Another study from Sudan found nearly identical results with NDM-producing BCC isolates [30]. Pathogens belonging to the BCC family have been identified as being responsible for a significant number of sepsis outbreaks in hospitals. Infection control authorities in clinical settings and hospitals received a wake-up call from these cases, prompting them to examine the issue and devise policies to deal with it. It is possible that the remaining cases were exposed to this organism as a result of inadequate hand hygiene practices as well as poor cleaning and disinfection procedures. Clinical and microbiologic follow-ups of cases that had been affected by the outbreak were used to evaluate how well infection control measures had been implemented. It was determined that the control methods were successful when there was a decrease in the number of infections and the mortality rate of patients. This outbreak was slowed down by to quick reporting to doctors, the deployment of infection control measures such as good hand hygiene,

proper washing and disinfection of NICU equipment, and tracking of patients who were infected with the illness.

Conclusion

This work demonstrates that the outbreak of NDM-producing *Burcholdreia cepacia complex* constituted a substantial hazard to neonatal patients. The majority of these infections reside in the NICU environment, which contributes to an increase in the mortality rate among neonates. The practice of isolating patients who are infected with BCC, conducting surveillance in the ward or hospital where it is occurring, practicing proper hand hygiene, cleaning medical equipment and the environment, educating medical personnel, and maintaining antibiotic stewardship are all standard methods used to manage and prevent the spread of BCC outbreaks. In the case that there is an epidemic of *Burkholderia cepacia*, it is essential to cooperate closely with healthcare professionals and adhere to their advice in order to effectively handle the situation and stop any further spread of the infection.

Summary Points

- *Burkholderia cepacia comples* (BCC) is a significant bacterium due to its association with various healthcare-associated infections and its potential to cause severe illnesses, particularly in vulnerable populations.
- 18 neonatal blood cultures were positive for BCC. 10/18 (55%) of the neonates were delivered via caesarean section.
- 10/18 (55.5%) neonates died who had BCC infection.
- 13/18 (72%) of the neonates had LONS, 5/18 (28%) EONS, 10 (55%) had low birth weight (≤2kg), and 15 (83%) had a hospital stay of ≥72 hours.

- Haematological findings revealed that 15 (83%) have high C-reactive protein (>6mg/dl), 14 (78%) have high white blood cell count (>10,000/cmm), and 8 (45%) have low platelet counts (50,000/cmm)
- All of the BCC isolates were found to be resistant to minocycline and chloramphenicol, followed by 14 (77.7%) to levofloxacin and 13 (72.2%) to ceftazidime, imipenem, and meropenem
- 13/18 (46.4%) carbapenemase-producing BCC tested positive for the blaNDM gene, according to the molecular identification of bla_{NDM}
- Environmental swabs (n=20) from NICU, 4/20 (20%) samples tested positive for BCC and these were found in an incubator, water sink, suction bottle, and suction catheter.
- All four environmental isolates were completely resistant to ceftazidime, imipenem, meropenem, levofloxacin, minocycline, and chloramphenicol. These isolates were positive for bla_{NDM}.

Financial and competing interest disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

This study was conducted with ethical approval from the Institutional Review Board (FMH-01-2021-IRB-865-M). Further, informed written consent for blood sample collection was obtained from the parents or guardians of the neonates.

Legends

Table 1 Clinical information of the neonatal septicemia patients

Table 2: Characteristics of *Burkholderia cepacia complex* sepsis in neonates

Table 3: Antimicrobial susceptibility profile of the blaNDM gene and clinically isolated BCC

Table 4: Environmental risk factors of BCC outbreak

Figure 1 Double disc synergy method (Left), and Modified Hodge's test (Right)
Figure 2 Molecular detection of bla_{NDM} in BCC

T1-to T9 test isolates, +ve control, -ve control, L: DNA ladder (100bp)

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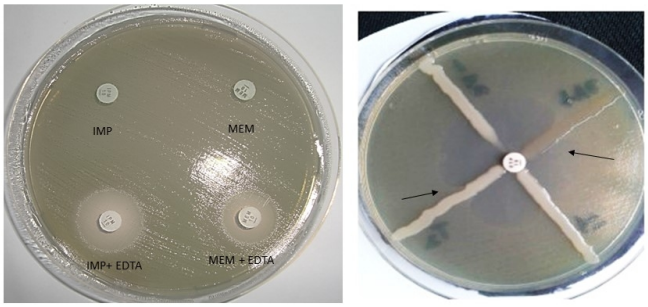
** The frequency of carbapenemase-producing bacilli was found to be high in hospital. NDM was found to be the most prevalent carbapenemase gene among clinical isolates. Close surveillance across all hospitals in Sudan is required.



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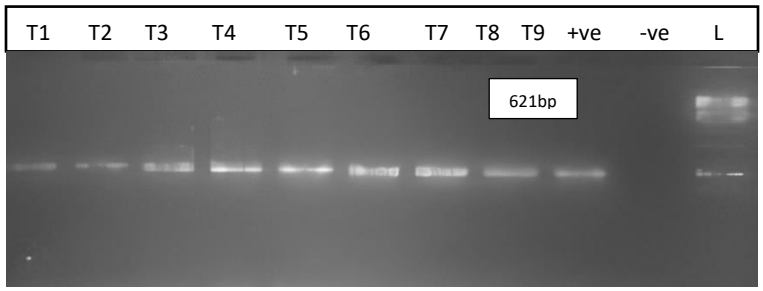

Clinical variables		**N (%)**
Gender	Male	11 (61%)
	Female	**7** (39%)
M to F Ratio		1.5:1
Age (day)	Range	1-29
	Mean	6.94
Mode of delivery	Vaginal delivery	8 (45%)
	Caesarean section	10 (55%)
Outborn/ Inborn	Out born	7 (39%)
	Inborn	11 (61%)
Gestational age	≥ 35 weeks	14 (78%)
	≤ 35 weeks	4 (22%)
EONS	-	5 (28%)
LONS	-	13 (72%)
Birth weight	≥ 2 kg	8 (45%)
	≤ 2 kg	10 (55%)
Premature rupture of membrane	Yes	2 (11%)
	No	16 (89%)
Hospital stays	≥ 72 hours	15 (83%)
	≤ 72 hours	3 (17%)
Mechanical ventilation	Yes	8 (45%)
	No	10 (55%)
Clinical conditions with neonatal sepsis		
Temperature	Hyperthermia	14 (78%)
	Hypothermia	4 (22%)
Convulsions	Yes	2 (11%)
	No	16 (89%)
Lethargy	Yes	10 (55%)
	No	8 (45%)
Respiratory rate	Normal	5 (28%)
	Tachypnea	10 (55%)
	Apnea	3 (17%)
Poor feeding	Yes	16 (89%)
	No	2 (11%)
Haematological factors with neonatal sepsis		
C-reactive protein	Non-reactive < 6 mg/dl	3 (17%)
	Reactive	15 (83%)
WBCs	Normal	4 (22%)
	High	14 (78%)
Platelets	Normal	10 (55%)
	Low	8 (45%)

EONS: Early-onset neonatal septicemia, LONS: Late-onset neonatal septicemia, WBCs: white blood cells.

Cases	Ward	Sample	Isolate	Age	Gender	Hospital stays	Predisposing factors	Outcome
Case 1	NICU	Blood	*B. cepacia*	1 day	Male	8 days	Preterm	Discharge
Case 2	NICU	Blood	*B. cepacia*	1 day	Female	30 days	Preterm	**Expired**
Case 3	NICU	Blood	*B. cepacia*	1 day	Male	11 days	Preterm	**Expired**
Case 4	NICU	Blood	*B. cepacia*	1 day	Male	14 days	Preterm	**Expired**
Case 5	NICU	Blood	*B. cepacia*	1 day	Female	8 days	Preterm	Discharge
Case 6	NICU	Blood	*B. cepacia*	2 days	Female	2 days	Preterm	Discharge
Case 7	NICU	Blood	*B. cepacia*	1 day	Female	4 days	PIH, Preterm	Discharge
Case 8	NICU	Blood	*B. cepacia*	3 days	Male	9 days	Syndromic	**Expired**
Case 9	NICU	Blood	*B. cepacia*	1 day	Male	2 days	Nil	Discharge
Case 10	NICU	Blood	*B. cepacia*	9 days	Female	4 days	Nil	Discharge
Case 11	NICU	Blood	*B. cepacia*	2 days	Male	16 days	Nil	**Expired**
Case 12	NICU	Blood	*B. cepacia*	29 days	Female	1 day	IUGR	**Expired**
Case 13	NICU	Blood	*B. cepacia*	1day	Male	9 days	PROM	Discharge
Case 14	NICU	Blood	*B. cepacia*	1 day	Female	2 days	TGDM	**Expired**
Case 15	NICU	Blood	*B. cepacia*	9 days	Male	4 days	IUGR	Discharge
Case 16	NICU	Blood	*B. cepacia*	18 days	Male	3 days	IUGR	**Expired**
Case 17	NICU	Blood	*B. cepacia*	18 days	Male	32 days	Syndromic	**Expired**
Case 18	NICU	Blood	*B. cepacia*	26 days	Male	11 days	Preterm	**Expired**

IUGR: Intrauterine growth retardation; PROM: preterm rupture of membrane; GDM: Gestational diabetes mellitus; PIH: Pregnancy-induced hypertension

Antimicrobials	**ATC code**	**WHO Classification**	**MIC Breakpoint (µg/ml)**	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16	Case 17	Case 18
Co-trimoxazole	J01EE02	**Access**	**≥4/76**	≥4/76	≥8/152	≤4/76	≥4/76	≥16/304	≤4/76	≥8/152	≥4/76	≥8/152	≥4/76	≤4/76	≥16/304	≥4/76	≥4/76	≥16/304	≥8/152	≤4/76	≥16/304
Chloramphenicol	J01BA01	**Access**	**≥32**	≥32	≥32	≥64	≥32	≥128	≥64	≥128	≥32	≥32	≥64	≥32	≥64	≥32	≥128	≥32	≥32	≥128	≥32
Ceftazidime	J01DD02	**Watch**	**≥32**	≥128	≥64	≥256	≥64	≤32	≥64	≥128	≤32	≥256	≤32	≥128	≥64	≥128	≥64	≥128	≤32	≥128	≤32
Levofloxacin	J01MA12	**Watch**	**≥8**	≥16	≥16	≤8	≥16	≥64	≥8	≥8	≥32	≤8	≥16	≥16	≤8	≥32	≥8	≤8	≥8	≥8	≥16
Imipenem	J01DH51	**Watch**	**≥16**	≥32	≥64	≥32	≥16	≤16	≥64	≥16	≤16	≥64	≤16	≥32	≥32	≥16	≥32	≥16	≤16	≥64	≤16
Meropenem	J01DH02	**Watch**	**≥16**	≥64	≥16	≥16	≥32	≤16	≥16	≥32	≤16	≥32	≤16	≥32	≥32	≥64	≥16	≥16	≤16	≥32	≤16
Minocycline	J01AA08	**Reserve**	**≥32**	≥32	≥32	≥64	≥32	≤32	≥32	≥64	≥32	≥128	≥32	≤32	≥32	≥64	≥32	≤32	≤32	≥32	≥32
blaNDM detected				**Positive**	**Positive**	**Positive**	**Positive**	Negative	**Positive**	**Positive**	Negative	**Positive**	Negative	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	Negative	**Positive**	Negative
Patients outcome				Discharge	**Expired**	**Expired**	**Expired**	Discharge	Discharge	Discharge	**Expired**	Discharge	Discharge	**Expired**	**Expired**	Discharge	**Expired**	Discharge	**Expired**	**Expired**	**Expired**

WHO: World Health Organisation, ATC code: Anatomical Therapeutic Chemical code.

This table shows that most of the BCC displayed increased resistance to AWaRe classes of antibiotics. Thirteen BCC have the NDM gene and 10 neonates had expired.

Antimicrobials	Ceftazidime	Co-trimoxazole	Levofloxacin	Imipenem	Meropenem	Minocycline	Chloramphenicol	blaNDM
MIC Breakpoint	**≥32**	**≥4/76**	**≥8**	**≥16**	**≥16**	**≥32**	**≥32**	
Incubator (n=1)	≥64	≥8/152	≥16	≥16	≥16	≥32	≥32	**Positive**
Sink drain (n=1)	≥64	≥4/76	≥16	≥16	≥32	≥32	≥32	**Positive**
Suction bottle (n=1)	≥64	≤4/76	≥8	≥64	≥16	≥32	≥64	**Positive**
The suction catheter (n=1)	≥128	≥16/304	≥64	≥16	≥16	≤32	≥32	**Positive**

Editorial Comments:

1. While I understand that you wish for your manuscript to be considered as a full research article, due to the limited sample size, word count and reference count, your manuscript would best suit a Short Communication. Please re-consider changing the article type upon re-submission.

Author response and action taken:
I have increased the length of the manuscript by increasing the words.

Commented [EP1]: Still only ~3000 words and low sample size.

2. Please further reduce the title to be less than 120 characters - a suggested title is 'Outbreak investigation of NDM-producing Burkholderia cepacia causing neonatal sepsis in Pakistan'. Please also make sure that the title in the manuscript, title page and on ScholarOne are the same.

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It has been corrected accordingly.

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3. Please reduce the length of your abstract is 120 words or less, and ensure that abbreviations are defined in full (e.g. NICU).

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4. Figures 1 and 2 are not very good quality - please could you edit these figures to improve their quality and size.

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5. It has been noted that reference annotations still have not been added. Please include reference annotations: up to 8 references should be chosen from your bibliography and highlighted as being '*' – of interest, or "**" – of considerable interest, with a brief sentence explaining why in each case.

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6. Certain sections of your article are still similar to previously published works. Please reword the highlighted sections in the attached document to avoid copyright issues.

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