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3 **Outbreak investigation of NDM-producing *Burkholderia cepacia* causing neonatal sepsis in**
4 **Pakistan**
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40 **Plain language summary**
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42 Neonatal septicemia (NS), or blood poisoning, is a dangerous illness in newborns. It is caused by
43 bacteria or other infections entering the blood and spreading. Pregnancy, labor, delivery and
44 exposure after birth can result in infection of the newborn. NS kills 700,000 babies worldwide,
45 mostly in low- and middle-income countries. *Burkholderia cepacia* complex (BCC) bacteria can
46 cause infections in people with weaker immune systems or other disorders. They are particularly
47 dangerous in hospitals as they can cause chronic lung problems. This study collected blood
48 samples from newborns with blood poisoning. Most samples that contained BCC were not
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3 susceptible to drugs. Four of the newborns carried the same bacteria, indicating that hospital staff
4 should practice hand washing and equipment and environmental cleaning to prevent the spread of
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6 the bacteria.
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10 11 12 **Abstract**

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14 **Aim:** To investigate the outbreak of *Burkholderia cepacia complex* (BCC), mortality,
15 antimicrobial resistance, and associated risk factors in the NICU. **Method:** 18 blood culture
16 samples from neonates and 20 swabs samples from different NICU surfaces were collected. The
17 VITEK 2 was used to confirm the isolates and generate the antibiogram. PCR was used to identify
18 *bla*NDM. **Results:** 18 samples tested positive for BCC, and 10/18 (55.5%) of the neonates died..
19 13/18 (72%) of the neonates had LONS, and 10 (55%) had low birth weight, Resistance to
20 minocycline and chloramphenicol was 100%, and 72.2% to meropenem. . 72.2% NDM gene was
21 found in neonates and was 20% from the environment. **Conclusion:** I Outbreak of NDM-
22 producing BCC resulting in high neonatal mortality.
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36 **Keywords:** Neonatal septicemia, NDM, antimicrobial resistance (AMR), *Burkholderia cepacia*
37 complex (BCC)
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43 **First draft submitted: 16 Mar 2023; Accepted for publication: 3 Aug 2023; Published**
44 **online: TBC**
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48 **Introduction:**

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51 An estimated 2.5 million newborns died within the first 28 days of life globally, due to infections
52 particularly sepsis, meningitis, and pneumonia with low-middle-income countries accounting for
53 nearly 80% of these deaths [1]. The risk of hospital-acquired infection (HAI) is incredibly high for
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3 neonates admitted to hospitals in low-resource settings, and it is associated with overcrowding and
4 understaffing, as well as lax infection control protocols [2]. Sepsis is defined as a potentially fatal
5 organ dysfunction syndrome caused by an abnormal host response to infections. When an infection
6 you already have sets off a series of events throughout your body, it results in sepsis. It is causing
7 infections typically begin in the gastrointestinal system, urinary tract, skin, or lungs. Sepsis can
8 quickly cause tissue damage, organ failure, and death if not treated promptly [3]. According to the
9 World Health Organization (WHO), septicemia accounts for 20% of all global deaths, with
10 children ≤ 5 accounting for half of all deaths [4]. Neonatal sepsis (NS) is divided into two types:
11 early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS). EONS is 0-7 days of
12 life and mostly acquired vertical infection (from mother to fetus) while LONS is 8-28 days and
13 linked with mainly horizontal transmission or HAI. *Klebsiella pneumoniae*, *Staphylococcus*
14 *aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, Group B
15 *Streptococcus* and *Burkholderia cepacia complex* (BCC) are the most notorious pathogens
16 responsible for HAI and outbreaks mainly in the neonatal intensive care units (NICU) [5, 6]. In
17 honour of its discoverer, BCC, formerly known as *Pseudomonas cepacia*, was given its genus in
18 1992. Presently, BCC includes seventeen closely related species (formerly known as genomovars).
19 BCC are the aerobic, catalase-producing, glucose-non-fermenting Gram-negative bacilli present
20 in soil and damp habitats that can survive and grow in nutrient-depleted water. BCC cause serious
21 illness in critically ill and immune-compromised patients and is not considered part of normal
22 human flora. It mainly affects immunocompromised children especially those who have a history
23 of malignancy, congenital heart disease and prematurity. [7]. Hospital outbreaks have been
24 reported because intravenous fluids, nebulizer solutions, contaminated disinfectants, medical
25 devices, and catheters are potential reservoirs for BCC [8]. Their infections are proving difficult

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

49 **Ethical consideration and clinical history of the patients**

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3 This cross-sectional study was conducted from February to May 2021 at the Department of
4 Pathology, Fatima Memorial Hospital (FMH) Lahore, with ethical approval from the Institutional
5 Review Board (FMH-01-2021-IRB-865-M). Further, informed written consent for blood sample
6 collection obtained from the parents or guardians of the neonates was obtained. Ethical approval
7 from each neonatal parent or guardian was obtained following the Declaration of Helsinki from
8 the Institutional Review Board, Fatima Memorial Hospital Lahore. The neonate in this study was
9 classified as being between 1 and 29 days old. Blood samples from neonates with clinical suspicion
10 of septicemia were collected from the NICU ward by the trained nursing staff and sent to FMH's
11 Microbiology laboratory for culture and sensitivity testing. The suspected neonates suffering from
12 heart rate >90 beats/mints, respiratory rate >20 breaths/mints, fever (>38°C) and hypothermia (<38
13 °C), leukocytosis, and leukopenia were included in the study. The hospital is a tertiary care facility
14 with 500 beds. The infection spread in the NICU and as a potential risk factor, the medical records
15 of all neonates suspected of septicemia were reviewed, including age, gender, date of hospital
16 admission, date of discharge, hospital stay, predisposing factors, gestational age, indoor and
17 outdoor temperature, pulse rate, heartbeat, weight, and patient outcome. Additionally,
18 haematological factors such as white blood cells, C-reactive protein, and platelets were taken into
19 account. A surveillance sampling was conducted by collecting the swabs from different surfaces
20 of the NICU from a tertiary care hospital in Lahore.

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 **Neonatal blood collection and the surveillance NICU ward**

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48 1-3ml blood was collected by trained nurses from clinically suspected neonates in Bactec/ALERT
49 PF blood culture bottles (Biomérieux, France) to confirm the laboratory diagnosis of sepsis. For
50 up to five days, the bottles were incubated and monitored using the Bactec/ALERT system
51 (Biomérieux, France). The key concept of the Bactec/ALERT is that when microorganisms are
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3 present in culture vials that cause them to metabolise the nutrients in the culture medium, they
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5 metabolise the nutrients and release carbon dioxide into the medium. A dye in the sensor at the
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7 bottom of the vial reacts to carbon dioxide. This alters the amount of light that the fluorescent
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9 material in the sensor can absorb. All positive blood culture bottles were subcultured on 5-10%
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11 sheep blood agar and MacConkey agar, and plates were incubated at 37 °C overnight for 24 hours
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15 aerobically.

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17 For NICU surveillance, 20 Amies transport swabs samples were collected from different areas of
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19 the NICU, including incubator humidifier (n=3), tap water (n=2), water sink (n=2), incubator
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21 surface (n=2), suction bottle (n=4), respiratory devices (n=4), and the suction catheter (n=3).
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23 Swabs samples were inoculated on the blood and MacConkey agar plates, and incubated for 24
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25 hours at 37 °C aerobically.
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29 Initially, the isolates were identified based on colony morphology, culture characteristics and
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31 Gram stain and further confirmation was carried out by the VITEK 2® system (bioMérieux SA,
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33 Marcy l'Etoile, France) confirmed both clinical and environmental bacteria using GN cards by
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35 utilizing 64 different biochemical substrates. The VITEK 2 systems employ advanced
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37 colorimetry™, an identifying technology that enables routine clinical isolate identification.
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39 Advanced colourimetry includes the following features, high interspecies discrimination. Multiple
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41 choice and misidentified species are uncommon.
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49 **Testing for antimicrobial susceptibility**

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52 Antimicrobial resistance is a major cause of clinical insensitivity to treatment and rapid
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54 progression to sepsis and septic shock. The antimicrobial susceptibility of *B. cepacia* isolates was
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3 determined using the Kirby-Bauer disc diffusion method and for the minimum inhibitory
4 concentration (MIC) by the VITEK 2 system, under Clinical and Laboratory Standard Institute
5 (CLSI) guidelines [14]. The VITEK® 2 antimicrobial susceptibility testing (AST) cards work
6 based on the broth microdilution minimum inhibitory concentration approach. The VITEK® 2
7 AST card is essentially a miniaturised, shortened, and automated version of the doubling dilution
8 process for microdilution-determined MIC. In short, 0.5McFarland bacterial suspension was made
9 and placed inside the VITEK 2 GN cards. The cards were placed inside the VITEK 2 system for
10 bacterial identification. The following antibiotics were used for the antibiogram such as
11 ceftazidime, co-trimoxazole, levofloxacin, minocycline, imipenem, and meropenem. The
12 interpretation of the antibiotics was determined as per CLSI guidelines.
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27 **Phenotypic detection of carbapenemase enzyme**

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29 Carbapenemase was detected using the Modified Hodge's test (MHT), as previously described
30 [15]. First, 0.5McFarland of ATCC 25922 *E. coli* control strain was prepared in sterile normal
31 saline. The isolates were lawned on the Mueller Hinton Agar (MHA) plate using a sterile swab. A
32 meropenem disc (10µg) was placed in the centre of the MHA plate and test organism, negative
33 control and positive control were streaked from the edge of the disc to the edge of the plate. The
34 MHA plate was incubated at 37 °C for 18-20 hrs in the aerobic incubator. After incubation, if
35 bacteria showed the clover leaf-like indentation the MHT was considered positive as described in
36 the CLSI guidelines.
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49 **Phenotypic detection of Metallo-β-lactamases (MBL)**

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51 The double disc synergy method was used to detect MBL as described previously [15]. In short,
52 MBLs require Zn⁺² as a cofactor for their enzymatic activity. By treating them with a chelating
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3 agent such as ethylene diamine tetraacetic acid (EDTA), their functional activity can be lost. On
4 the MHA plate, 0.5McFraland bacterial suspension was lawned. Two meropenem (10µg) and two
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8 ertapenem (10µg) discs were placed, and one meropenem and ertapenem disc was treated with
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10 0.5M EDTA solution. Bacteria were MBL producers if the EDTA discs had a zone of inhibition
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12 greater than 5mm when compared to non-EDTA discs.
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15 **Molecular identification of *bla*_{NDM-1}**

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

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49 Further, the amplicon DNA was shipped to Euroffin UK for Sanger-based DNA sequencing. 15µl
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51 of purified DNA with the concentration of 10ng/µl was added into the sterile Eppendorf with 2µl
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53 of NDM forward and NDM reverse primers with a concentration of 10µM. The DNA sequence
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3 data was obtained and analysed by different bioinformatics tools. First, the DNA sequence was
4 analysed by the Genious software (<http://www.geneious.com/>). Further, the data was analysed by
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6 sequence similarity and region locus similarity using National Center for Biotechnology
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8 Information (NCBI) database and Basic Locus Alignment Search Tool (BLAST)
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10 (<http://www.ncbi.nlm.nih.gov/>). The aligned sequences were compared with other NDM variants
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12 sequences in the NCBI database.
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16 17 **RESULTS**

18 19 **Patient's clinical characteristics**

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21 The *Burkholderia cepacia complex* was found in all 18 neonatal blood cultures in the NICU of the
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23 tertiary care hospital. The majority of these isolates were recovered from males (11; 61%) rather
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25 than females (7; 39%), with a male-to-female ratio of 1.5:1 The ages ranged from 1 to 29 days,
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27 with a mean of 6.94 days. Ten (55%) of the neonates were delivered via caesarean section, with
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29 the majority of these being born in the hospital (11; 61%). 13 (72%) of the neonates had LONS, 5
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31 (28%) EONS, 10 (55%) low birth weight (≤ 2 kg), and 15 (83%) had a hospital stay of ≥ 72 hours.
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33 With septicemia, 14 (78%) of the neonates have hyperthermia, 10 (55%) have tachypnea and
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35 lethargy, and 16 (89%) have poor feeding. Furthermore, haematological findings revealed that 15
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37 (83%) have high C-reactive protein (>6 mg/dl), 14 (78%) have high white blood cell count
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39 ($>10,000$ /cmm), and 8 (45%) have low platelet counts (50,000/cmm) (Table 1).
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46 47 **Characteristic of neonatal septicemia**

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49 In the NICU of the tertiary care hospital, BCC were recovered from all neonatal septicemia. The
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51 most concerning finding was the deaths of 10/18 (55.5%) neonates who had BCC infection.
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53 Among these, 4/10 (40%) neonates died with preterm, 2/10 (20%) with syndromic and 2/10 (20%)
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3 with intrauterine growth retardation (IUGR). The age ranges from 1-29 days with a mean age was
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5 6.94 days on average. 8 (44.4%) patients stayed in the NICU for one to seven days, 7 (38.8%) for
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7 seven to fourteen days, and 3 (16.6%) for more than fourteen days. In addition, 2 (11.1%) patients
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9 have the syndrome, followed by 3 (16.6%) patients with IUGR, and 8 (44.4%) patients with
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11 preterm birth. Further, 12 (66.6%) had EONS ranging from 1 to 3 days of life and 6 (33.3%) had
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13 LONS with an age range from 9 to 29 days of life (Table 2).
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19 **Phenotypic confirmation of carbapenemase and MBL production**

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21 The phenotypic test was employed for the validation of carbapenemase and MBL enzymes.
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23 Phenotypic The studies of the carbapenemase and MBL revealed that 13/18 (72.2%) BCC
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25 produced carbapenemase using the MHT, and 12/18 (66.6%) produced MBL using the double disc
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27 synergy method (Figure 1). The BCC isolates were subsequently examined for the molecular
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29 confirmation of bla_{NDM}.
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34 **Profile of antimicrobial resistance**

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36 The Kirby-Bauer disc diffusion method was used to perform the preliminary screening of the
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38 antimicrobial susceptibility profile, and the VITEK 2 system was used to perform the subsequent
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40 confirmation. The AST results displayed that all of the BCC isolates were found to be resistant to
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42 minocycline ($\geq 32\mu\text{g/ml}$) and chloramphenicol ($\geq 32\mu\text{g/ml}$), followed by 14 (77.7%) to
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44 levofloxacin ($\geq 8\mu\text{g/ml}$) and 13 (72.2%) to ceftazidime ($\geq 32\mu\text{g/ml}$), imipenem ($\geq 16\mu\text{g/ml}$), and
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46 meropenem ($\geq 16\mu\text{g/ml}$). According to the WHO's classification of antibiotics (AWaRe), there
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48 were two antibiotics that belonged to the "Access" group (co-trimoxazole and chloramphenicol),
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50 four antibiotics that belonged to the "Watch" group (ceftazidime, levofloxacin, imipenem, and
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52 meropenem), and one antibiotic that belonged to the "Reserve" group (minocycline).
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Molecular detection of *bla*_{NDM} carried by BCC isolates

According to the molecular identification of *bla*_{NDM}, the results of the molecular profile showed that 13/18 (46.4%) carbapenemase-producing BCC tested positive for the *bla*_{NDM}. 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 $\mu\text{g/ml}$), imipenem (≥ 16 $\mu\text{g/ml}$), meropenem (≥ 16 $\mu\text{g/ml}$), levofloxacin (≥ 8 $\mu\text{g/ml}$), minocycline (≥ 32 $\mu\text{g/ml}$), and chloramphenicol (≥ 32 $\mu\text{g/ml}$), according to the antimicrobial susceptibility profile.

Molecular identification of *bla*_{NDM} containing BCC in environmental 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 *bla*_{NDM}. 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

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3 Infection control measures including education of hospital health workers, severe infection control
4 measures, education of healthcare workers, and decontamination of the NICU's medical equipment
5 including ventilators, baby incubators, baby cots, suction tubes, ambo bags, and ventilators.
6
7 Infection control methods include education of hospital health workers. Following the completion
8 of a surveillance study of the NICU, the ward was fumigated and decontaminated. Additionally,
9
10 training was provided on hand hygiene, cleaning, cleaning of the patient's surrounds, donning clean
11 aprons while dispensing clean materials, and donning clean aprons while preparing clean materials
12 for the production of new patient beds. Other training topics included the correct donning and
13 doffing of gloves, the correct disposal of bio waste, and training on personal protective equipment.
14
15 During the staff work, the committee nurse participated in observations and provided criticism. In
16 addition, education was offered to the personnel regarding the significance of BCC as well as BCC
17 infections. Following the implementation of infection control measures, the NICU did not have
18 any BCC strains in its samples.
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35 **DISCUSSION**

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37 *Burkholderia cepacia complex* is the notorious nosocomial pathogen prevalent in NICU that can
38 be found in antiseptics, nebulizer solutions, ventilator tubing, and indwelling catheters. Invasive
39 medical devices and the incorrect use of broad-spectrum antibiotics are usually linked to prolonged
40 hospital stays for various medical or surgical operations. These stays are frequently caused by the
41 patient requiring multiple interventions. An outbreak case was defined as a neonate who had a
42 clinical suspicion of sepsis (fever, tachycardia, tachypnea, leukocytosis, or leukopenia, with or
43 without hypotension) and one or more BCC-positive blood culture results. In the current
44 investigation, all BCC were obtained from neonatal septicemia in the NICU. The majority of the
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3 patients had LONS, had a low birth weight (less than 2 kilograms), and had spent less than 72
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5 hours in the hospital. Two of the clinical signs that occurred in neonates were hyperthermia and
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7 poor eating. Septicemia was proven by the elevated level of C-reactive protein as well. These
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9 findings are in line with data that were only recently reported from an epidemic of BCC in
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11 Peshawar, Pakistan's neonatal intensive care unit. [16]. Another study from India documented 12
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13 cases of neonates in the NICU with septicemia in a tertiary care hospital [17]. A Gambian study
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15 documented the sequential outbreak of BCC and MDR *K. pneumoniae* in the NICU of a tertiary
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17 care hospital [4]. Further, an outbreak of neonatal septicemia in the surgical unit was also reported
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19 in another study from India [18]. All of these factors, in addition to BCC's inherent ability to
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21 survive on moist surfaces within the environment, contribute to their effectiveness as infections in
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23 healthcare facilities. The most pressing problem with these organisms is that the virulence of sepsis
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25 is not significantly different from the virulence of other Gram-negative organisms, and given the
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27 length of our patients' stays in the hospital, the organism may become resistant to many antibiotics.
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29 The identical BCC strains were cultivated from every feasible source, including the incubator, the
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31 respirator, and the surfaces in the NICU ward. These are some of the potential factors that led to
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33 the BCC outbreak. Several studies [19-22] have found contamination of BCC in the hospital
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35 environment and during the manufacturing of different products and after the product has been
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37 opened. According to a recent outbreak report, the contaminated ultrasound probe gel used in
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39 ultrasound procedures resulted in *B. cepacia* bacteremia in patients [23]. Further, a Turkish study
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41 found the presence of BCC in mouthwash solutions in tertiary care hospitals [5]. Our isolates'
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43 minimum inhibitory concentration determination revealed multidrug resistance. In the present
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45 study, our investigation demonstrated resistance to ceftazidime while previous studies reported
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47 sensitivity to ceftazidime and other cephalosporins. The BCC recovered from patients and the
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3 NICU environment displayed the same biological characteristics and antimicrobial-resistant
4 profile. The previous data suggested that BCC are intrinsically resistant to various classes of
5 antibiotics including aminoglycosides, quinolones, first, second and third-generation of
6 cephalosporins and left limited treatment options. The medicines useful against BCC were
7 levofloxacin, meropenem, co-trimoxazole, ceftazidime, and minocycline as per CLSI guidelines.
8 However, the combination of the antibiotic is considered effective for drug-resistant BCC [24, 25].
9
10 Worryingly, 10/18 (55.5%) neonates died as a result of BCC infection in this study. Previously
11 studies reported neonatal mortality due to BCC infections globally. A Korean study reported 41%
12 neonatal mortality [26], 25.9% in Singapore [27] and 50% mortality in Malaysia [28]. NDM are
13 β -lactam genes that produce resistance to a variety of antibiotic classes, including carbapenems.
14 We identified 13/18 (72.2%) NDM-producing BCC cases and 4/20 (20%) of the environmental
15 samples were NDM producers. The NDM-producing BCC clinical isolates were documented in
16 an Indian study [29]. Another study from Sudan found nearly identical results with NDM-
17 producing BCC isolates [30]. Pathogens belonging to the BCC family have been identified as being
18 responsible for a significant number of sepsis outbreaks in hospitals. Infection control authorities
19 in clinical settings and hospitals received a wake-up call from these cases, prompting them to
20 examine the issue and devise policies to deal with it. It is possible that the remaining cases were
21 exposed to this organism as a result of inadequate hand hygiene practices as well as poor cleaning
22 and disinfection procedures. Clinical and microbiologic follow-ups of cases that had been affected
23 by the outbreak were used to evaluate how well infection control measures had been implemented.
24 It was determined that the control methods were successful when there was a decrease in the
25 number of infections and the mortality rate of patients. This outbreak was slowed down by quick
26 reporting to doctors, the deployment of infection control measures such as good hand hygiene,
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3 proper washing and disinfection of NICU equipment, and tracking of patients who were infected
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5 with the illness.
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8 **Conclusion**

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

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39 • *Burkholderia cepacia complex* (BCC) is a significant bacterium due to its association with
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41 various healthcare-associated infections and its potential to cause severe illnesses,
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43 particularly in vulnerable populations.
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- 46 • 18 neonatal blood cultures were positive for BCC. 10/18 (55%) of the neonates were
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48 delivered via caesarean section.
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- 51 • 10/18 (55.5%) neonates died who had BCC infection.
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- 53 • 13/18 (72%) of the neonates had LONS, 5/18 (28%) EONS, 10 (55%) had low birth weight
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55 (≤ 2 kg), and 15 (83%) had a hospital stay of ≥ 72 hours.
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- 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 *bla*_{NDM} 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.

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3 **Legends**
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6 **Table 1 Clinical information of the neonatal septicemia patients**
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9 **Table 2: Characteristics of *Burkholderia cepacia complex* sepsis in neonates**
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12 **Table 3: Antimicrobial susceptibility profile of the bla_{NDM} gene and clinically isolated**
13 **BCC**
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17 **Table 4: Environmental risk factors of BCC outbreak**
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20 **Figure 1 Double disc synergy method (Left), and Modified Hodge's test (Right)**

21 **Figure 2 Molecular detection of bla_{NDM} in BCC**
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24 **T1-to T9 test isolates, +ve control, -ve control, L: DNA ladder (100bp)**
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13 a further worrying development for India, which already has high levels of antibiotic
14 resistance.
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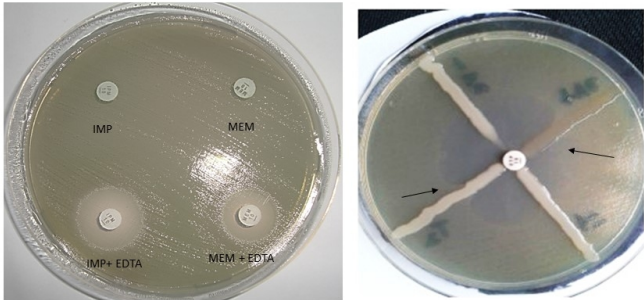
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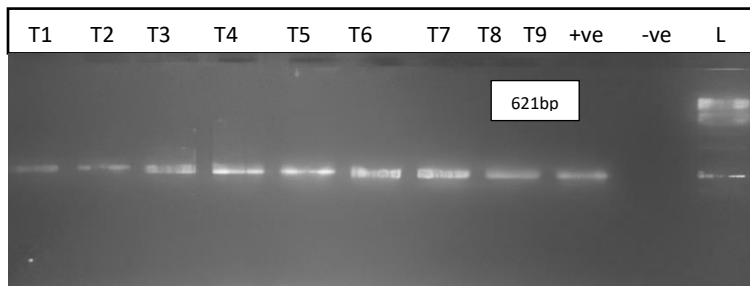
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25 ** The frequency of carbapenemase-producing bacilli was found to be high in hospital. NDM was
26 found to be the most prevalent carbapenemase gene among clinical isolates. Close
27 surveillance across all hospitals in Sudan is required.
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Clinical 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	<i>B. cepacia</i>	1 day	Male	8 days	Preterm	Discharge
Case 2	NICU	Blood	<i>B. cepacia</i>	1 day	Female	30 days	Preterm	Expired
Case 3	NICU	Blood	<i>B. cepacia</i>	1 day	Male	11 days	Preterm	Expired
Case 4	NICU	Blood	<i>B. cepacia</i>	1 day	Male	14 days	Preterm	Expired
Case 5	NICU	Blood	<i>B. cepacia</i>	1 day	Female	8 days	Preterm	Discharge
Case 6	NICU	Blood	<i>B. cepacia</i>	2 days	Female	2 days	Preterm	Discharge
Case 7	NICU	Blood	<i>B. cepacia</i>	1 day	Female	4 days	PIH, Preterm	Discharge
Case 8	NICU	Blood	<i>B. cepacia</i>	3 days	Male	9 days	Syndromic	Expired
Case 9	NICU	Blood	<i>B. cepacia</i>	1 day	Male	2 days	Nil	Discharge
Case 10	NICU	Blood	<i>B. cepacia</i>	9 days	Female	4 days	Nil	Discharge
Case 11	NICU	Blood	<i>B. cepacia</i>	2 days	Male	16 days	Nil	Expired
Case 12	NICU	Blood	<i>B. cepacia</i>	29 days	Female	1 day	IUGR	Expired
Case 13	NICU	Blood	<i>B. cepacia</i>	1 day	Male	9 days	PROM	Discharge
Case 14	NICU	Blood	<i>B. cepacia</i>	1 day	Female	2 days	TGDM	Expired
Case 15	NICU	Blood	<i>B. cepacia</i>	9 days	Male	4 days	IUGR	Discharge
Case 16	NICU	Blood	<i>B. cepacia</i>	18 days	Male	3 days	IUGR	Expired
Case 17	NICU	Blood	<i>B. cepacia</i>	18 days	Male	32 days	Syndromic	Expired
Case 18	NICU	Blood	<i>B. cepacia</i>	26 days	Male	11 days	Preterm	Expired

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

Anti-microbials	ATC code	WHO Classification	MIC Breakpoint ($\mu\text{g/ml}$)	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
Cotrimoxazole	J01EE02	Access	$\geq 4/76$	$\geq 4/76$	$\geq 8/152$	$\leq 4/76$	$\geq 4/76$	$\geq 1/6304$	$\leq 4/76$	$\geq 8/152$	$\geq 4/76$	$\geq 8/152$	$\geq 4/76$	$\leq 4/76$	$\geq 1/304$	$\geq 4/76$	$\geq 4/76$	$\geq 1/6304$	$\geq 8/152$	$\leq 4/76$	$\geq 1/6304$
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
Mincycline	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

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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.

Author response and action taken:

It has been corrected accordingly.

Commented [EP2]: Not done - still too long, and needs to be the same on ScholarOne.

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

Author response and action taken:

It has been corrected accordingly.

Commented [EP3]: 122 words.

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

Author response and action taken:

It has been corrected accordingly.

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.

Author response and action taken:

It has been corrected accordingly.

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.

Author response and action taken:

It has been rephrased accordingly.

7. Please copy your ethical disclosure from the manuscript onto the title page.

Author response and action taken:

It has been done.

8. Please further revise the Plain Language Summary for a broader audience e.g., with no scientific background. For assistance in drafting a PLS, please use the EMA medical term simplifier and the Flesch Kincaid readability calculator, links to which can be found in our Author Guidelines. Please consider asking a friend/family member with a non-scientific background to proof your PLS. Should it be useful, please use the resources below to help simplify the text. Flesch calculator assesses the readability of the lay abstract and you should aim for a reading age of 6th grade (11-12 years old). The higher the Flesch Reading Ease Score the more accessible it will be for non-specialist readers: <https://goodcalculators.com/flesch-kincaid-calculator/>

These dictionaries may help source alternative words: <https://apps.lib.umich.edu/medical-dictionary/><https://www.smartpatients.com/glossary>

Author response and action taken:

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It has been included.

Commented [EP4]: Too long - needs to be 250 words max.

9. Please check all authors and affiliations both in your title page and the ScholarOne record. Currently co-author Alia Batool is listed in your manuscript but not in Scholar One. You will not be able to edit the author list, so please provide an email address for Alia Batool and I can add them on your behalf.

Author response and action taken:

It has been corrected.

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