Supplementary material 1

Appendix A

Phenotypic detection of extended-spectrum beta-lactamases producing pathogenic *Escherichia coli* with the Double Disk Synergy Test

All positive PEC isolates were tested for ESBL production by the Double Disc Synergy Test (DDST) using a disc of cefotaxime (CTX-30 µg) and cefotaxime-clavulanate (CTX/CV-30/10 µg) (Mast Group Ltd, United Kingdom) according to the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines. A spread culture of each bacteria strain was made on a Mueller-Hinton agar (MHA) (Oxoid Ltd, United Kingdom) plate from a prepared 0.5 McFarland suspension in saline (0.85%) (DMP-NHLS, South Africa). An impregnated disc for CTX-30 µg and CTX/CV-30/10 µg was aseptically placed on each agar plate 2.5 cm apart and incubated (Vacutec, South Africa) at 37°C for 16 h to 18 h. Bacterial strains of *Escherichia coli* ATCC® 25922 and *Klebsiella pneumoniae* ATCC® 700603 were used for quality control. The zones of inhibition of each isolate were interpreted according to the guidelines (CLSI, 2020).

Appendix B

Phenotypic detection of carbapenemase-producing pathogenic *Escherichia coli* with the modified carbapenem inactivation method

The modified carbapenem inactivation method (mCIM) test was performed on all PEC positive isolates. In brief, a 1 µL loopful of each PEC bacteria strain cultured overnight on blood agar plates (Oxoid Ltd, United Kingdom) and incubated (Vacutec, South Africa) at 37°C for 16 h to 18 h was emulsified in 2 mL of tryptic soy broth (TSB) (Oxoid Ltd, United Kingdom) in a 5 mL culture tube (Merck Group, Germany)

using a sterile inoculating loop as was recommended by the CLSI (2020) guidelines. The bacterial suspension was vortexed for 10 to 15 secs, thereafter, a 10 µg meropenem (MEM) disk (Mast Group Ltd, United Kingdom) was immersed into each TSB bacterial suspension (Oxoid Ltd, United Kingdom) using sterile forceps. All bacterial suspensions were incubated (Vacutec, South Africa) at 37 °C for 4 h ± 15 min for carbapenem inactivation. Prior to completion of the 4 h incubation period, a 0.5 McFarland suspension in saline (0.85%) (DMP-NHLS, South Africa) was prepared using the mCIM indicator organism (*Escherichia coli* ATCC® 25922), a carbapenem-susceptible strain and inoculated on MHA (Oxoid Ltd, United Kingdom) plates as recommended by the CLSI (2020) guidelines. Subsequently, the MEM disks were removed from the TSB bacterial suspension using a sterile 10 µL inoculating loop, dragging the loop along the inside edge of the tube to expel excess liquid from the disks. The disks were aseptically applied to the MHA (Oxoid Ltd, United Kingdom) agar plates and incubated (Vacutec, South Africa) in an inverted position at 37°C for 18 h to 24 h in ambient air. All results were interpreted according to the CLSI (2020) guideline. The mCIM result was termed positive and the PEC strain was regarded as a carbapenemase producer if the zone size was between 6 mm and 15 mm, negative if the zone size was ≥19 mm and intermediate (also termed positive) if pinpoint colonies were present within a zone size of 16 mm to 18 mm.

Appendix C

Rapid colistin NP test for detection of polymyxin resistance in pathogenic *Escherichia coli*

All PEC isolates recovered were tested for polymyxin resistance using the rapid colistin NP test as previously described (Nordmann *et al.*, 2016). A colistin sulfate

(Sigma-Aldrich, USA) stock solution (1.280 µg/mL) was prepared as described by the CLSI (2020). A 200 µg/mL colistin working solution was prepared from the colistin sulfate (Sigma-Aldrich, USA) stock solution for use in the colistin NP test. Subsequently, a 250 mL of rapid colistin NP solution was prepared by dissolving 6.25 g of cation-adjusted Mueller-Hinton broth (CA-MHB) powder (Becton, Dickinson and Company, USA) and 0.0125 g of phenol red powder (Sigma, USA) in 225 mL of distilled water. By adding drops of 1 mol/L hydrochloric acid (HCl) (Saarchem, Wadeville, South Africa), the pH of the solution was adjusted to 6.7 and thereafter autoclaved (EINS-Sci, South Africa) at 121°C for 15 min. After autoclaving, the solution was allowed to cool to ambient temperature (25°C $\pm$ 5°C) before adding 25 mL of a filtered 10% anhydrous D-glucose solution (Amresco, LLC, Ohio, USA). To perform the colistin NP test, the colistin sulfate working solution prepared was added to the colistin NP solution in a sterile glass tube (Merck Group, Germany) to bring the final concentration of colistin sulphate in the colistin NP solution to 5 µg/mL just before inoculation into the microtitre plate (Eppendorf, Germany). Each microtitre plate (Eppendorf, Germany) comprises of a growth control well and a colistin NP test solution well for each tested isolate. A 3.0 to 3.5 McFarland suspension of each PEC bacterial isolate was prepared in saline (0.85%) (DMP-NHLS, South Africa) and 50 µL of each was inoculated into both the growth control and the colistin NP test solution well on the microtitre plates. Colistin-resistant (*Proteus mirabilis* ATCC 25933) and colistin-susceptible (*E. coli* ATCC 25922) isolates were used as controls for each test. Plates were incubated (Vacutec, South Africa) and uncovered at 37°C in an aerobic atmosphere. The plates were examined for spontaneous colour changes after 10 minutes and every hour for a maximum of 4 hours. Colour changes were

assessed by two separate readers after all plates had been incubated for the entire 4 hours.

Appendix D

Antimicrobial susceptibility testing and isolate identification of pathogenic *Escherichia coli*

A 0.5 McFarland suspension of each PEC bacterial isolate was prepared in saline (0.85%) (DMP-NHLS, South Africa) from fresh colonies of overnight grown cultures on 5% sheep blood agar (Oxoid Ltd, United Kingdom) incubated (Vacutec, South Africa) at 37°C for 16 h to 18 h. The bacterial suspension was dispensed into dried inoculators and inoculated unto the MIC panels (110-115 µL) using the RENOK® system, loaded unto the WalkAway 40 plus Microscan system (Beckman Coulter Inc, California, USA) and MIC readings taken 18 h to 24 h later according to the automated reading standardized by the manufacturer. The minimum inhibitory concentration (MIC) of each isolate was tested for amikacin, ampicillin, aztreonam, cefotaxime, cefoxitin, ceftazidime, cefuroxime, ciprofloxacin, colistin, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, nalidixic acid, nitrofurantoin, norfloxacin, piperacillin/tazobactam, tigecycline, trimethoprim/sulfamethoxazole, tobramycin, cefepime, ampicillin/sulbactam and amoxicillin/clavulanate antimicrobials. The concentration for each tested antimicrobial required to kill 90% of the bacterial strains (MIC_{90}) were compared to the respective breakpoints (BP). The *E. coli* ATCC 25922 was included in the analysis as quality control.