Risk factors for, and molecular epidemiology and clinical outcomes of, carbapenem- and polymyxin-resistant Gram-negative bacterial infections in pregnant women, infants, and toddlers: a systematic review and meta-analyses

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Abstract

In the following systematic review and meta-analyses, we report several conclusions about resistance to carbapenem and polymyxin last-resort antibiotics for treating multidrug-resistant bacterial infections among pregnant women and infants. Resistance to carbapenems and polymyxins is increasing, even in otherwise vulnerable groups such as pregnant women, toddlers, and infants, for whom therapeutic options are limited. In almost all countries, carbapenem-/polymyxin–resistant *Klebsiella pneumoniae, Escherichia coli*, and *Acinetobacter baumannii* infect and/or colonize neonates and pregnant women, causing periodic outbreaks with very high infant mortalities. Downregulation of plasmid-borne bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla_{IMP}, bla_{VIM}, bla_{GES-5}, and *ompK35/36* in clonal strains accelerates the horizontal and vertical transmissions of carbapenem resistance among these pathogens. New Delhi metallo-β-lactamase (NDM)–positive isolates in infants/neonates have been mainly detected in China and India, while OXA-48–positive isolates in infants/neonates have been mainly detected in Africa. NDM-positive isolates in pregnant women have been found only in Madagascar. Antibiotic therapy, prolonged hospitalization, invasive procedures, mechanical ventilation, low birth weight, and preterm delivery have been common risk factors associated with carbapenem/polymyxin resistance. The use of polymyxins to treat carbapenem-resistant infections may be selecting for resistance to both agents, restricting therapeutic options for infected infants and pregnant women. Currently, low- and middle-income countries have the highest burden of these pathogens. Antibiotic stewardship, periodic rectal and vaginal screening, and strict infection control practices in neonatal ICUs are necessary to forestall future outbreaks and deaths.

Keywords: carbapenem; polymyxin; colistin; carbapenemase; antibiotic resistance; pregnantwomen; infants; neonates

Introduction

Resistance to antimicrobials remains a major challenge to public health, veterinary medicine, agriculture, and aquaculture globally, owing to the limited or no therapeutic option available to treat multidrug-resistant (MDR) infections.[1-5] To avoid depleting our antibiotics arsenals

against infectious pathogens, certain agents are reserved as last-line drugs to fight difficult-to-treat infections.[6-8] Currently, carbapenems, polymyxins (colistin), and tigecycline are used as reserved agents for MDR infections.[9, 10] However, the continual use of carbapenems and polymyxins to treat bacterial infections resistant to penicillins, cephalosporins, and carbapenems is increasing the rate of resistance to these last-resort antibiotics.[2, 4, 9, 11] The emergence of carbapenem-resistant (CR) and/or polymyxin-resistant (PR) MDR infections is thus limiting therapeutic options and increasing hospital stay, healthcare costs, morbidity, and mortality.[12-14] Subsequently, the WHO has classified CR Gram-negative bacteria (CR-GNB) as critical priority pathogens for which novel antibiotics are urgently needed.[15, 16]

Pregnant women, infants, and toddlers (under 5 years old) are a vulnerable population with regard to bacterial infections because of their unbalanced and less matured immune system, respectively, and the limited therapeutic options available to them.[17-19] Hence, the presence of MDR and CR and/or PR infections in pregnant women, infants, and toddlers makes these patients more susceptible to the ramifications of the infection.[13, 19, 20] Whereas several studies have reported on pregnant women colonized with CR-GNB than those infected by it, there is a risk of colonized pregnant women transferring these CR-GNB to their newborns.[19, 21-24] It is, therefore, not surprising that more studies report on CR-GNB– and PR Gram-negative bacteria (PR-GNB)–infected neonates than colonized ones.[20, 23, 25, 26] Furthermore, there have been abundant reports of clonal and polyclonal CR-GNB outbreaks in neonatal intensive care units (NICUs) globally, claiming the lives of several neonates as a result.[18, 27-29] This highlights the likely susceptibility of neonates to CR- and PR-GNB infections with their associated morbidity and mortality.[17-20, 30]

Notably, CR- and PR-GNB carry other antibiotic resistance genes (ARGs) on mobile plasmids, transposons, integrons, integrative conjugative elements, and insertion sequences (ISs) that facilitate the horizontal transmission of these ARGs between species and clones.[13, 20, 31-33] Specifically, extended-spectrum β-lactamases (ESBLs), fluoroquinolones, aminoglycosides, tetracycline, sulphamethoxazole-trimethoprim, and macrolide ARGs are also found in CR- and PR-GNB. Hence, under antibiotic selection pressure, MDR plasmids and clones proliferate, leading to outbreaks.[10, 27, 34] It is thus not surprising that most neonatal CR Enterobacterales (CRE) infections occur in NICUs during clonal outbreaks in which the isolates harbor multiple ARGs on mobile plasmids.[20, 25, 26, 30, 35, 36] Analyses of the data obtained from this study confirm this observation.

Methods

Evidence before this review

A thorough review of the literature showed the absence of a review addressing carbapenem and polymyxin resistance among pregnant women, toddlers, infants, and neonates. Only one review addressed carbapenem resistance among Gram-negative bacteria (GNB) in children,[14] while another only addressed CRE-causing neonatal sepsis in China.[37] Thus, this is the first work to systematically review and statistically analyze the literature on carbapenem and polymyxin resistance among pregnant women and infants.

Literature and database search strategy

All literature published in English were searched on Medline/PubMed, Web of Science, ScienceDirect, HINARI, Cochrane Library electronic databases, and Google Scholar using

the following search words: "Gram-negative bacteria," "epidemiology," "prevalence," "incidence," "risk factors," "determinant," "associated factors," "carbapenem," "carbapenem resistance," "colistin," "polymyxin resistance," "carbapenem-resistant Gram-negative bacteria," "colistin-resistant Gram-negative bacteria," "carbapenem resistance determinants," "carbapenem resistance genotypes," "carbapenem resistance mechanisms," "colistin resistance determinants," "colistin resistance genotypes," "colistin resistance mechanisms," "infants," "neonates," "children," and "pregnant women." These search words were further paired with each other in a factorial fashion, and search strings were implemented using "AND" and "OR" Boolean operators. "OR" was used between "colistin" and "polymyxin" as in "colistin OR polymyxin." No other filters were employed, and the search was carried out from the earliest dates (without any cutoff) up to September 30, 2020. The first two authors undertook the literature search and title and abstract screening, and resolved outstanding discrepancies by consensus, using the inclusion and exclusion criteria.

Inclusion and exclusion criteria

All articles addressing the following were included: (1) molecular testing of carbapenem- and polymyxin (colistin)-resistant GNB isolated from pregnant women, toddlers, and/or infants; (2) phenotypic antimicrobial sensitivity testing of carbapenem- and polymyxin (colistin)-resistant GNB isolated from pregnant women, toddlers, and/or infants; (3) epidemiology (incidence, prevalence, risk factors, and/or clinical outcomes) of carbapenem and/or polymyxin (colistin) resistance in pregnant women, infants, and/or toddlers (<5 years old); and (4) articles on pregnant women and infants with carbapenem- or polymyxin (colistin)-resistant infections or colonization. Articles reporting on carbapenem- and/or polymyxin (colistin)-resistant GNB isolated from nonpregnant women and/or children older than 5 years old were excluded.

Statistical analyses

Data on isolation country, year of isolation, carbapenem and polymyxin resistance profiles, GNB species and clones, carbapenem and polymyxin resistance mechanisms, age, sex, clinical history, colonization or infection, clinical history, previous and current medications, clinical outcomes, mortality rate, and risk factors were extracted from the included articles and populated into Microsoft Excel. The significance of the association between countries and resistance determinants, countries and species, countries and clones, and age and clinical outcome was computed using the one-sample t-test and the Wilcoxon signed-rank test with GraphPad® Prism 9.1.0 (221); P values of <0.05 were defined as significant. Row statistics and associated graphs for each dataset (see Table S4, online only) were also undertaken using GraphPad Prism 9.1.0 (221) to determine the means, medians, and standard deviations. Maps showing the global distribution of carbapenem and polymyxin resistance mechanisms, as well as global mortality rates of toddlers, infants, and neonates infected with CR-GNB, were also drawn using Microsoft Excel. All charts were drawn with Microsoft Excel.

Further analyses were undertaken to compute isolation rates, prevalence, resistance rates, and mortality rates per country (Tables S1–S3, online only). This systematic review and meta-analyses was performed using the PRISMA guidelines and criteria.

Results

Included studies and samples

A total of 1427 articles were retrieved from the various databases, that is, PubMed ($n = 1304$), Web of Science ($n = 37$), ScienceDirect ($n = 39$), HINARI ($n = 27$), Cochrane Library electronic database ($n = 11$), Google Scholar ($n = 9$), and other sources ($n = 42$) (Fig. S1 and Dataset 1, online only), of which 73 studies from 28 countries worldwide were finally included for the qualitative and quantitative analyses (Fig. S1, online only). Studies describing carbapenem resistance in toddlers, infants, and neonates ($n = 65$ studies) were from 24 countries, involved 49,154 toddlers, infants, and neonates (<5 years), and resulted in the isolation of 11,441 GNB cultures with a 23.3% isolation rate (Table S1, online only). Carbapenem resistance in pregnant women ($n = 7$ studies) was reported from six countries among 1892 pregnant women; this resulted in 285 GNB cultures and an isolation rate of 15.1% (Fig. 1; and Table S2, online only). Included studies on polymyxin resistance ($n = 7$) were mainly in toddlers, infants, and neonates ($n = 16,556$), with one being from a pregnant woman;[38] 1345 GNB cultures were obtained, yielding an isolation rate of 8.1% (Fig. 1; and Fig. S2 and Table S3, online only).
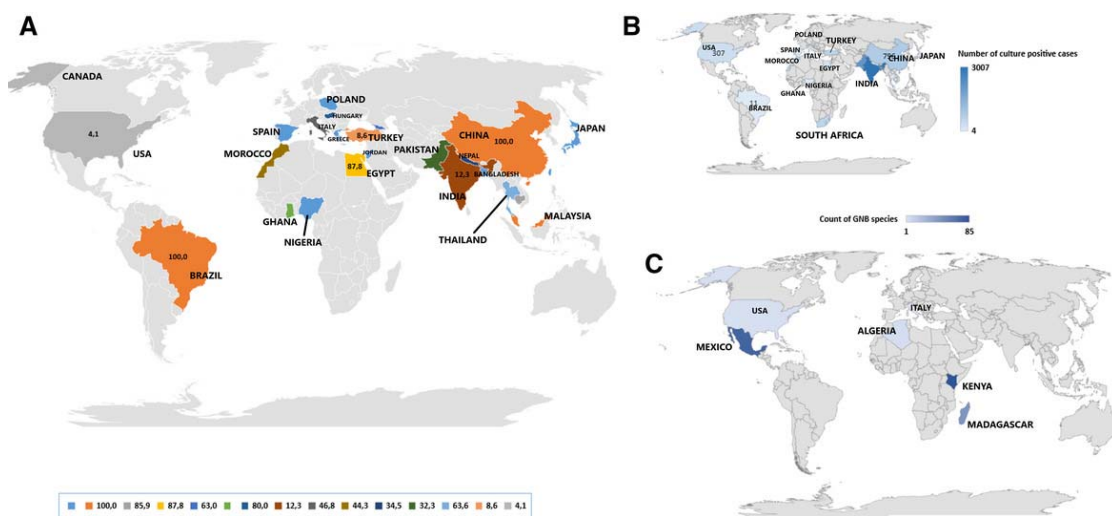

Figure 1. The distribution and burden of Gram-negative bacteria isolated from neonates, infants, and pregnant women. (A) The global distribution of carbapenem-resistant Gram-negative bacteria (CR-GNB) isolated from toddlers, infants, and neonates. (B) Gram-negative bacterial isolations reported globally. (C) The regions of Earth CR-GNB bacteria isolated from pregnant women.

Fifty studies on toddlers, infants, and neonates involved carbapenem resistance infections, 11 involved colonization by CR-GNB, and four involved both infection and colonization. At least 22 studies out of the 50 describing GNB infections in neonates involved sepsis cases (Table S1, online only). Notably, all the studies on PR-GNB in toddlers, infants, and neonates also involved infection cases with no colonizations (Table S3, online only). However, six out of the seven studies on CR-GNB in pregnant women were mainly colonization cases with no infections, with only one study being an infection case in the United States (Table S2, online only). There were higher GNB isolations from India, Bangladesh, Pakistan, Morocco, China, Turkey, South Africa, the United States, and Cambodia than from the other countries (Fig. 1; and Fig. S2, online only).

Age and clinical outcome

Of the 14,732 toddlers, infants, neonates, and pregnant women infected/colonized with CR-/PR-GNB, 78.63% ($n = 11,584$) were neonates aged between 0 and 28 days, 3.86% ($n = 568$) were infants aged between 1 and 60 days, 8.01% ($n = 1180$) were toddlers between the ages of 1 day and 1 year, while 0.14% ($n = 21$) were from toddlers aged between 7 days and 36 months (Fig. 2; and Table S3, online only). Notably, 1379 pregnant women aged between 16 and 45 years of age were infected/colonized with CR-PR-GNB within the study period, with those aged between 16 and 45 years ($n = 1020$) and 23 and 32 years ($n = 356$) forming the majority of those infected/colonized.
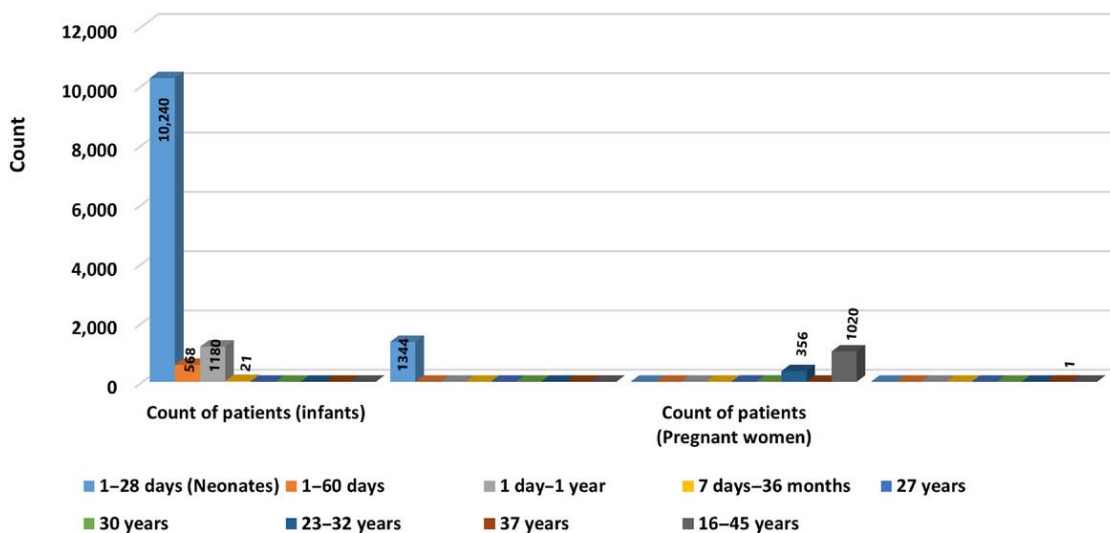

Figure 2. Age distribution of patients infected or colozined by carbapenem- and polymyxin-resistant Gram-negative bacteria (CR-/PR-GNB). A large proportion of the patients studied in the included studies were within 0–28 days (neonates). Pregnant women aged between 16 and 45 years and babies above 1 year old were fewer in numbers.

However, none of the pregnant women who were infected/colonized with CR-PR-GNB died, while 1157 neonates (aged between 0 and 28 days) and 20 infants (below 1 year old) died of CR-GNB infections. Particularly, all the recorded deaths were due to CR-GNB, specifically from India ($n = 1002$ deaths), Egypt ($n = 32$ deaths), Nepal ($n = 27$ deaths), Pakistan ($n = 24$), and Thailand ($n = 23$), with none being due to PR-GNB (Fig. 3; Fig. S3 and Table S4, online only). Nevertheless, the mortality rates were higher in China (36.8%), Brazil (27.3%), Egypt (14.5%), Thailand (11.6%), and Hungary (10%) than in India (4.1%), which had the highest number of actual infant deaths. Interestingly, there was no statistically significant association between the countries and deaths as well as between age and death, although most deaths occurred in one country and in neonates (Fig. 3; and Fig. S3, online only).
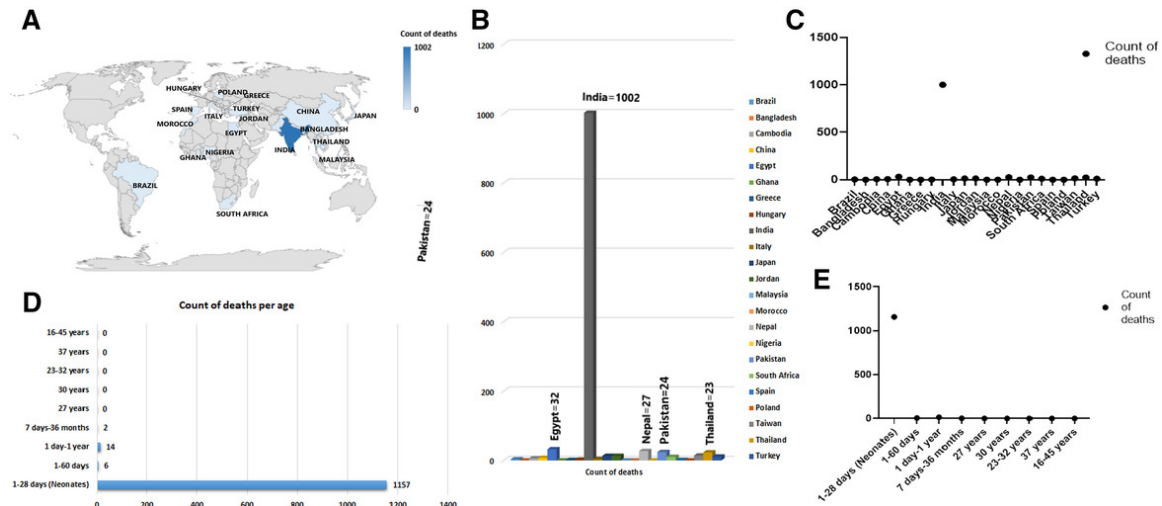
Figure 3. Global mortalities and ages of patients affected by carbapenem-resistant Gram-negative bacteria (CR-GNB). (A) The global burden of death resulting from CR-GNB infection among infants and neonates. (B) The count of deaths among infants, neonates, and toddlers infected by CR-GNB. (C) The row statistics graph showing the standard deviation (SD) of the death counts per countries. (D) Death counts per age of patient. (E) The row means statistics of D, showing the age group with the highest SD from the mean.

Species associated with carbapenem and polymyxin resistance

On a per-country basis, *Escherichia coli* (*n* = 3611 isolates), *Klebsiella pneumoniae*/spp. (*n* = 2507 isolates), *Acinetobacter baumannii*/spp. (*n* = 1343 isolates), *Pseudomonas aeruginosa*/spp. (*n* = 822 isolates), and *Enterobacter* spp. (cloacae) (*n* = 380) isolates were the most dominant species causing infections or colonization in toddlers, infants, neonates, and pregnant women, with *Proteus* spp. (*n* = 107), *Citrobacter* spp. (*n* = 57), *Serratia* spp. (*n* = 31), *Salmonella* spp. (*n* = 10), and *Morganella* spp. (*n* = 2) being some of the less common species (Fig. 4; and Fig. S4, online only). Although these species differed with reference to prevalence per country, *E. coli*, *K. pneumoniae*, and *A. baumannii* were the most common in almost all countries, while *P. aeruginosa* was highly prevalent in Italy, Nepal, the United States, Turkey, and Kenya.

As shown in Figure 4A and 4B, there were fewer species diversity among pregnant women than in infants (23 genera in infants/neonates compared with seven in pregnant women), with *E. coli* (73.68%) and *K. pneumoniae* (16.49%) being very dominant among pregnant women (Table S4 and Fig. S4, online only). *A. baumannii* was absent among pregnant women. Notwithstanding, *A. baumannii* was the commonest species (72.73%) implicated in polymyxin resistance among infants and neonates. *E. coli* (4.55%), *K. pneumoniae* (16.67%), and *P. aeruginosa* (1.52%) were less implicated in polymyxin resistance (Fig. 4). There were statistically significant associations between *K. pneumoniae/*spp. (*P* = 0.002), *A. baumannii/*spp. (*P* = 0.0321), *P. aeruginosa/*spp. (*P* = 0.0415), and *Enterobacter* spp. (*cloacae*) (*P* = 0.0229), and carbapenem resistance among neonates and infants, with *K. pneumoniae* having a lower *P* value than the rest. Furthermore, *K. pneumoniae* alone had a significant association with carbapenem resistance among pregnant women. Yet, no significant association was observed between any of the species and polymyxin resistance (Fig. 4; and Table S4, online only).
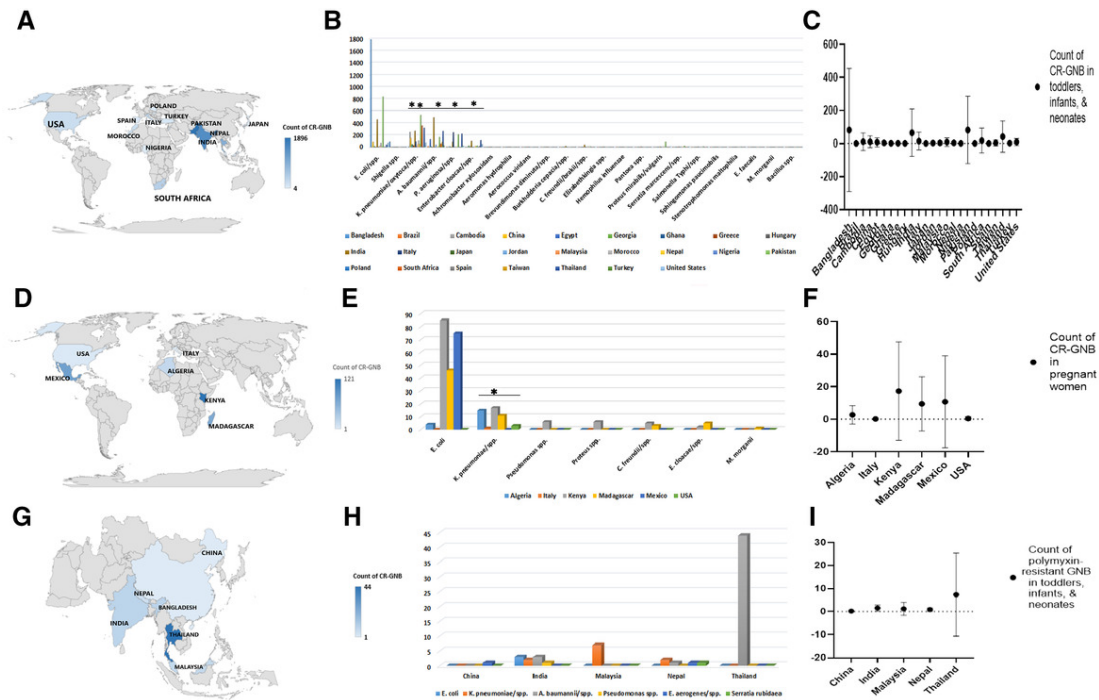
Figure 4. The global distribution and count of carbapenem- and polymyxin-resistant Gram-negative bactieral (CR-/PR-GNB) species. (A, D, and G) The maps showing regions with CR-/PR-GNB among neonates/infants and pregnant women. (B, E, and H) The counts of CR-/PR-GNB per country among neonates/infants and pregnant women. (C, F, and I) The row statistics of B, E, and H showing the standard deviations of GNB counts from the mean. *P values of <0.05.

The global distribution of CR-/PR-GNB presents an interesting observation. Southeast Asia, namely, India ($n = 1501$), Bangladesh ($n = 1882$), Pakistan ($n = 1896$), and Thailand ($n = 966$), reported higher cases of CR-GNB among neonates and infants than other countries in Africa, Europe, and the Americas (Fig. 4A). Among pregnant women, however, the highest CR-GNB cases were reported in Africa, viz., Kenya ($n = 121$), Madagascar ($n = 66$), Algeria ($n = 19$), and Mexico ($n = 75$) (Fig. 4D). Interestingly, the geographical location of PR-GNB among infants and neonates mirrored that of CR-GNB in neonates, as all the PR-GNB cases were reported from Southeast Asia: China ($n = 1$), India ($n = 9$), Malaysia ($n = 7$), Nepal ($n = 5$), and Thailand ($n = 44$). Thailand, therefore, had higher cases of both CR- and PR-GNB (Fig. 4; and Table S4, online only).

Clones associated with carbepenem and polymyxin resistance

The dominant clones of the various species were mainly localized within countries, with few clones being observed across countries. For instance, only *K. pneumoniae* ST11 (in India, China, and multiple settings), *K. pneumoniae* ST15 (India, China, and Nepal), and *K. pneumoniae* ST17 (Ghana and China) were observed across countries (Fig. 5; and Fig. S5, online only). *E. coli* ST410 ($n = 86$) was very predominant among infants and neonates in China, while *K. pneumoniae* ST1878 ($n = 10$) and ST13 ($n = 4$) were commonly found in pregnant women in Algeria. Relatively few studies characterized the CR-GNB isolates to determine their clonality, while others used PFGE or REP-PCR, limiting an effective international clonal analysis (Fig. 2). Yet, the various clones identified within the countries were mostly involved in outbreaks in NICUs.[39-44] The clonality of species with polymyxin resistance was not determined in the included studies. No significant statistical association

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was obtained between any of the clones and carbapenem resistance or between the clones and a country (Fig. 5; and Fig. S5, online only).
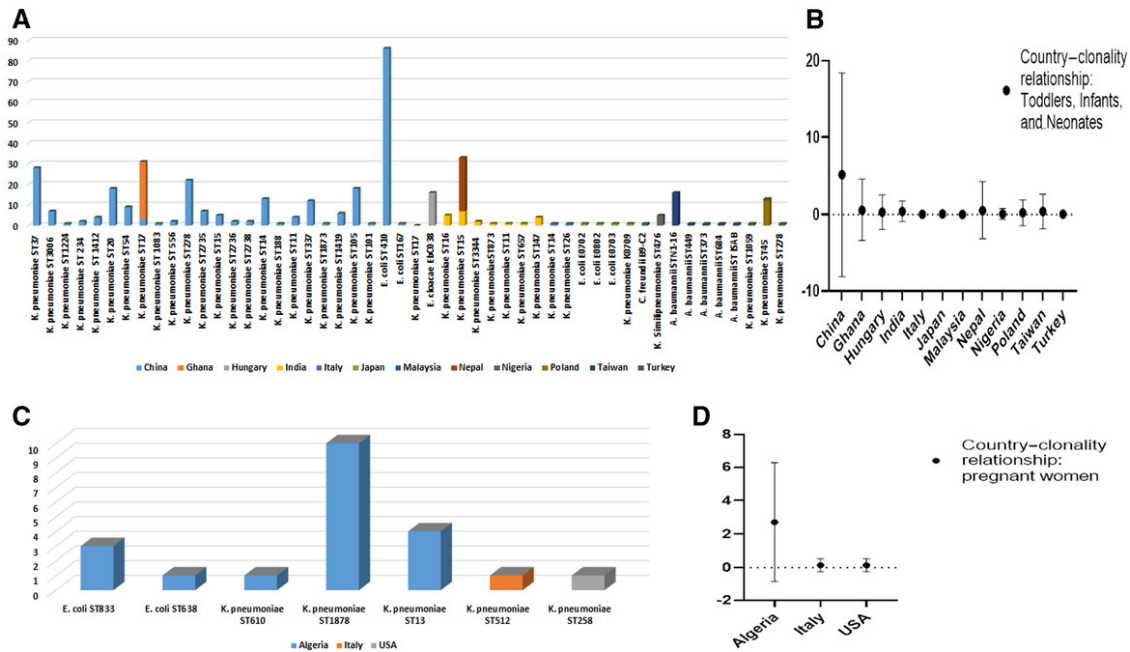

Figure 5. Clones of Gram-negative bacteria infecting/colonizing infants, neonates, and pregnant women. (A and C) The counts of the clones of the various species infecting or colonizing toddlers, infants, and neonates and pregnant women, respectively. (B and D) The row statistics of A and C showing the standard deviation (SD) of the clones.

Carbapenem and polymyxin resistance rates

Carbapenem resistance among toddlers, infants, and neonates in the various countries was substantial, with eight countries having a carbapenem resistance rate of more than 50% among infants and neonates, and three countries having a 100% carbapenem resistance rate among isolates from pregnant women (Fig. 6; Fig. S6 and Table S4, online only). This is observable in Figure 6A among infants: Brazil (54.5%), Egypt (80.3%), Ghana (100%), Greece (100%), Japan (100%), Nigeria (71.4%), Poland (100%), Taiwan (100%), and Turkey (72.6%). China (100%) and Thailand (42.6%) had higher polymyxin resistance rates among neonates and infants, while Algeria, Italy, and the United States had 100% carbapenem resistance rates in GNB from pregnant women. In terms of absolute figures, India ($n = 877$), Pakistan ($n = 294$), Egypt ($n = 139$), Thailand ($n = 125$), Taiwan ($n = 102$), and the United States ($n = 100$) had higher CR-GNB. In pregnant women, Algeria (19 CR-GNB), Madagascar (11 CR-GNB), and Mexico (11 CR-GNB) recorded the highest number of CR-GNB colonizations (Fig. S6, online only). The carbapenem resistance rates among the neonates/infants ($P = 0.0002$) and pregnant women ($P = 0.042$) were statistically significant, but that of polymyxin was not (Fig. 6).
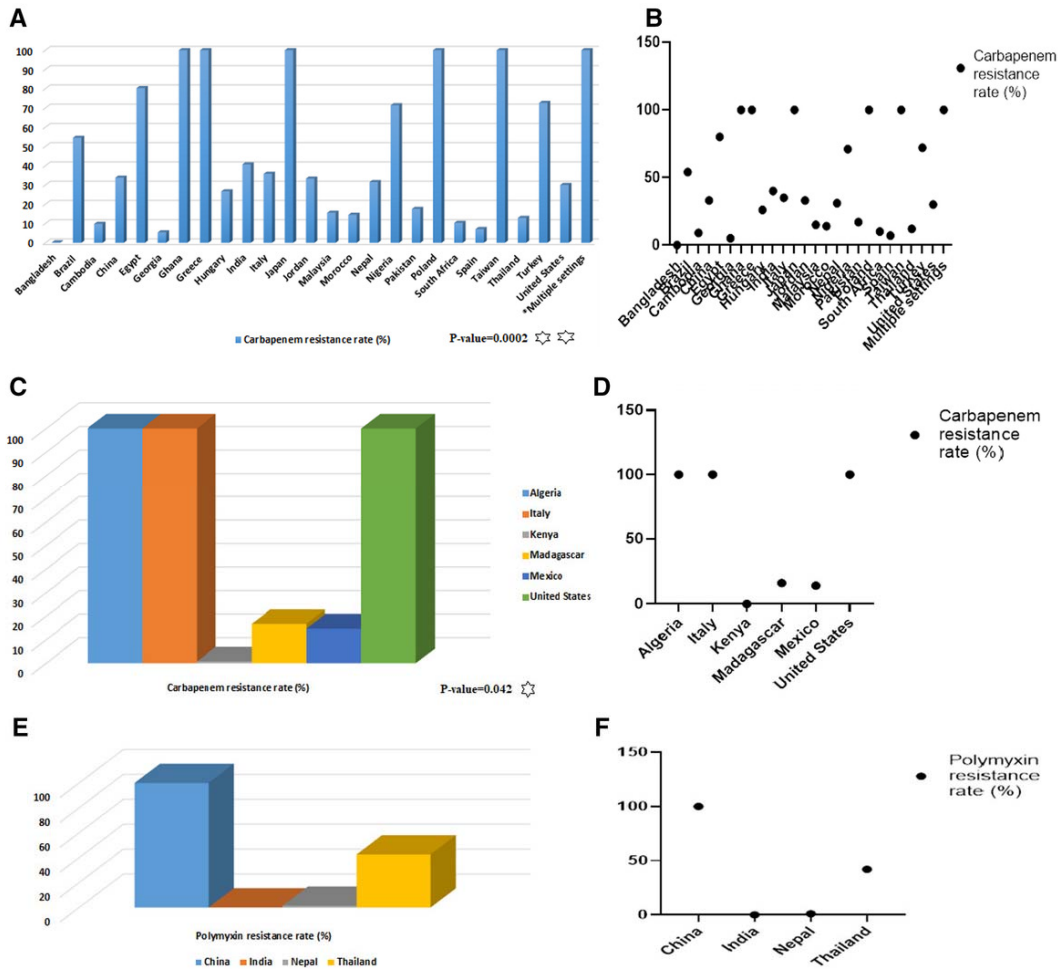
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Figure 6. Carbapenem and polymyxin resistance rates. (A, C, and E) The resistance rates of carbapenems and polymyxins among neonates, infants/toddlers, pregnant women, and infants/neonates, respectively. (B, D, and F) The row statistics of A, C, and E showing their standard deviations from the mean resistance rates.

Carbapenem and polymyxin resistance determinants

Carbapenem resistance among CR-GNB in toddlers, infants, neonates, and pregnant women was mainly mediated by carbapenemases in all countries, with few reports of noncarbapenemases, such as the loss of OmpK35 and OmpK36 porins (Italy), penicillin-binding protein mutations (PBP3) (Malaysia), OXA-1 β-lactamase (China), and SoxS and MarA efflux regulators (Malaysia), mediating carbapenem resistance. GES-5 (Poland), IMP (Japan and China), and VIM (Italy, Hungary, and Morocco) carbapenemases were mainly dominant in single or few countries or regions (such as the Mediterranean region), while New Delhi metallo-β-lactamase (NDM), KPC, and OXA-48–like variants were common in many countries among toddlers, infants, neonates, and pregnant women. Polymyxin resistance mechanisms were largely not described in the included studies, except in one study by Naha *et al*., in which overexpression of *acrB*, *tolC*, *ramA*, and *soxS* was found to mediate colistin resistance,[35] and in another study by Zeng *et al*., in which *mcr-1* was found in an *Enterobacter* (now *Klebsiella*) *aerogenes* isolate (Fig. 7; Fig. S7 and Table S4, online only).
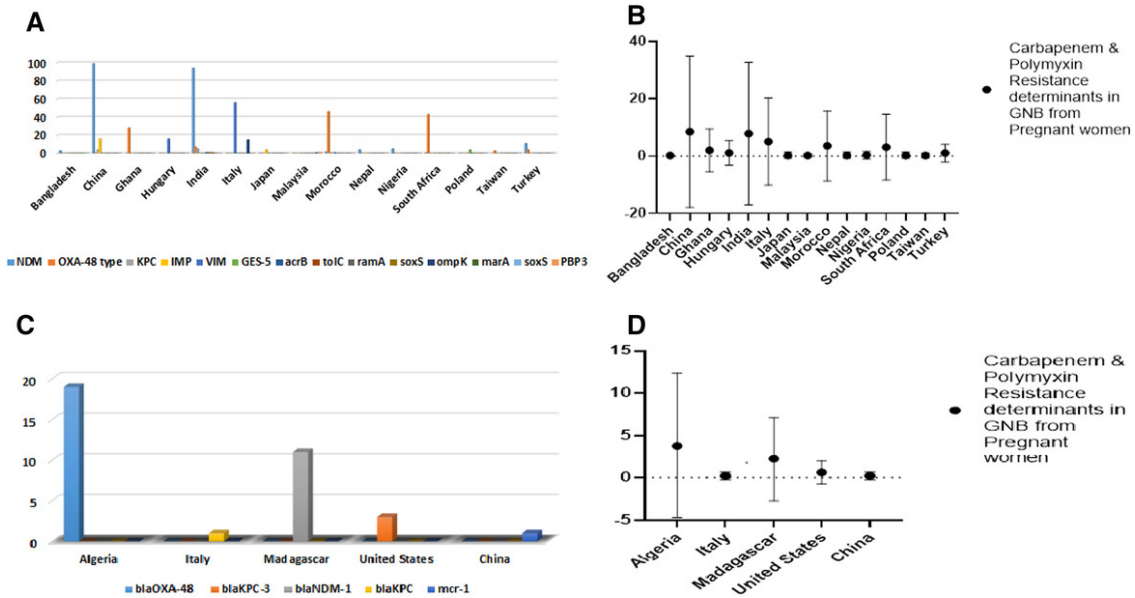
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Figure 7. Carbapenem and polymyxin resistance mechanisms. (A and C) Carbapenem and polymyxin resistance mechanisms in neonates, infants/toddlers, and pregnant women, respectively. (B and D) The row statistics of A and C, showing their standard deviations from the mean of resistance mechanisms.

NDM was the commonest carbapenemase among infants and neonates ($n = 219$) and the second most common among pregnant women ($n = 11$), while OXA-48–like carbapenemases were the second most detected in infants/neonates ($n = 131$) and the most common among pregnant women ($n = 19$). VIM ($n = 73$) and IMP ($n = 20$) carbapenemases were also common among GNB from infants and neonates but were absent in pregnant women, while KPC was relatively less prevalent among GNB from both infants/neonates ($n = 10$) and pregnant women ($n = 4$). It is worth noting that OXA-48 was only reported from Algeria ($n = 19$), while NDM was only detected in Madagascar ($n = 11$) among pregnant women. Furthermore, OXA-48 among isolates in infants/neonates was mostly reported from Africa: Ghana ($n = 28$), Morocco ($n = 46$), and South Africa ($n = 43$). Also, NDM was mainly detected in isolates from China ($n = 99$) and India ($n = 94$). There was no significant association between any carbapenemase or resistance mechanism and the reporting country, infants/neonates, and pregnant women (Fig. 7; and Table S4, online only).

Risk factors and mortality rates

Risk factors associated with CR- and PR-GNB infections and colonization differed slightly between countries, with factors, such as previous and/or long hospitalization, previous and/or ongoing antibiotic therapy, invasive procedures, such as cesarean section, intubation, and catheterization, preterm and low birth weight infants, and mechanical ventilation being common risk factors in almost all countries. Whereas PR-GNB–associated mortalities were not reported, CR-GNB–associated mortalities were above 10% in Brazil, China, Egypt, Hungary, and Thailand and noticeably lower than 10% in India, Nepal, Turkey, Pakistan, Italy, and Cambodia (Fig. 8; and Fig. S8, online only).
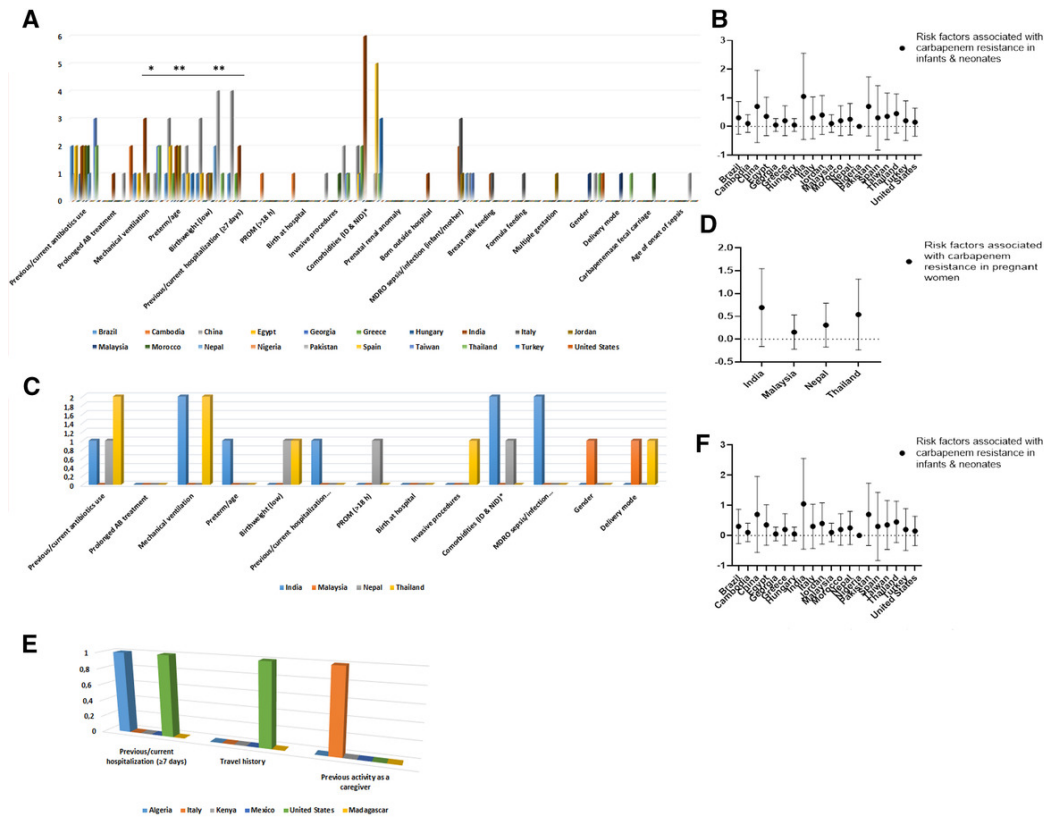
Figure 8. Risk factors associated with carbapenem and polymyxin resistance. (A, C, and E) Risk factors, respectivley, associated with carbapenem and polymyxin resistance in neonates, infants, toddlers, and pregnant women. (B, D, and F) The row statistics of A, C and E, showing their standard deviations from the mean of risk factors. * and ** denote risk factors that are statistically significant: *P* value of <0.05.

As can be seen from Figure 8 and Table S4 (online only), comorbidities (*n* = 22), previous and/or current antibiotic use/therapy (*n* = 18), preterm delivery (*n* = 16), lower birth weight (*n* = 15), mechanical ventilation (*n* = 11), infection with an MDR GNB (*n* = 9), and previous/prolonged hospitalization (*n* = 8) were the commonest risk factors associated with being infected with CR-GNB among neonates/infants across several countries. Similarly, previous/current antibiotic use among pregnant women (*n* = 4) and mechanical ventilation (*n* = 4) were more common risk factors among pregnant women. Previous/current hospitalization, travel history, and previous activity as a caregiver were the sole risk factors reported for PR-GNB among neonates/pregnant women. Delivery method, gender, breast or formula feeding, and fecal carriage of carbapenemases were less common risk factors. Of all the factors, mechanical ventilation (*P* = 0121), preterm delivery (*P* = 0.013), and low birth weight (*P* = 0.0074) were found to be statistically significant (Fig. 8; and Table S4, online only).

Discussion

CR- and/or PR-GNB are common in neonates and pregnant women in several countries as either infections or colonization, threatening the lives of the affected patients as CR- and PR-GNB are associated with longer hospitalization and higher morbidities and mortalities.[29, 45-47] Worryingly, many of the studies involved in this review reported on clonal and polyclonal outbreaks and infections of CR- or PR-GNB among neonates in NICUs or colonization of

CR-GNB in pregnant women involving pathogenic GNB, such as *A. baumannii, E. coli, Enterobacter* spp.*, K. pneumoniae*, and *P. aeruginosa* (Tables S1–S4, online only). In most of these CR-GNB, carbapenenemases including NDM, OXA-48, KPC, VIM, IMP, and GES-5 were found on mobile plasmids within transposons, ISs, and integrons.

In this work, we show that *K. pneumoniae* was significantly associated with carbapenem resistance among infants/neonates and pregnant women (Fig. 4), while *A. baumannii, P. aeruginosa*, and *Enterobacter* spp/*cloacae* were only significantly associated with carbapenem resistance in infants/neonates. Nevertheless, *E. coli*, which was the most dominant species among the reported CR-GNB isolates in these analyses, was not statistically significant in its association with carbapenem or polymyxin resistance. This could be due to the larger number of *E. coli* isolates from only three South Asian countries: Bangladesh ($n = 1789$), India ($n = 463$), and Pakistan ($n = 837$). Thus, although *E. coli* was more common than *K. pneumoniae*, it is mostly restricted to South Asia, while *K. pneumoniae* is generally distributed in many countries globally, making *K. pneumoniae* more likely to mediate the transmission of carbapenem resistance among pregnant women, infants, and neonates. The predominant role of *K. pneumoniae*, followed by *E. coli, Enterobacter* spp., and *A. baumannii*, in spreading carbapenem and polymyxin resistance is well noted in the literature.[1, 2, 9, 48]

It is worth mentioning that important GNB pathogens, such as *Salmonella enterica* Typhi ($n = 10$), *Shigella* spp. ($n = 90$), and *Vibrio cholerae* ($n = 0$), were less reported, albeit other Enterobacterales species, such as *Serratia marcescens/*spp. ($n = 30$)*, Citrobacter freundii/*spp. ($n = 49$), and *Proteus mirabilis/vulgaris* ($n = 99$), were also implicated in carbapenem resistance in infants/neonates. Hence, clinicians must be on a lookout for all these species as potential bearers of carbapenemases, particularly those that are intrinsically resistant to polymyxins: the Proteeae and *Serratia* spp.[49-51]

Notably, CR *A. baumannii, E. coli*, and *K. pneumoniae* harboring mobile plasmid–borne carbapenemases cause outbreaks in neonates with sepsis, pneumonia, and other infections in NICUs in several countries worldwide, resulting in very high mortalities (Fig. 3; and Table S1, online only).[17, 42, 52-54] For instance, CR *A. baumannii* outbreaks were reported by Lee *et al*., Sultan and Seliem, Indhar *et al*., Al-lawama *et al*., Thatrimontrichai *et al*., Kumar *et al*., and Tekin *et al*. in Taiwan, Egypt, Pakistan, Jordan, Greece, India, and Turkey, respectively, killing several infants.[19, 54-59] *E. coli* neonatal infections and outbreaks were reported in Japan with an IMP-11 carbapenemase,[36] in China with nine NDM-5–producing ST410 strains[44] and NDM-5–positive ST167,[33] in Turkey with an OXA-48–producing strain,[42] and in India with NDM-1–positive clonal strains from the body of toddlers, infants, neonates, and the ward environment.[41] Comparatively, outbreaks in NICUs involving *K. pneumoniae* were globally reported, involving NICU infections, colonizations, mobile plasmid–borne *bla*NDM, *bla*KPC, *bla*OXA-48, *bla*IMP, *bla*VIM, and *bla*GES-5 carbapenemases, and very high mortalities, making *K. pneumoniae* the most deadly and common neonatal MDR pathogen.[13, 18, 25, 27, 28, 31, 35, 39, 40, 42, 43, 52, 53, 60-66]

Similarly, PR-GNB infections were also common in the same species, viz., *A. baumannii, E. coli, K. pneumoniae, Enterobacter* spp., and *Pseudomonas* spp., although reports on outbreaks were either associated with carbapenem resistance[34, 35] or absent. Hence, outbreaks involving CR-GNB that were also polymyxin resistant could also be referred to as PR-GNB outbreaks, which makes such infections very difficult to manage.[1, 2, 4, 9] The largest collection of PR-GNB was recorded in Malaysia from preterm infants in an NICU in 2016, eight of

which were CR *K. pneumoniae*.[34] A colistin-resistant *C. freundii* isolate was subsequently isolated from the stool of a preterm infant in the same hospital in Malaysia, suggesting the persistence of PR-GNB in that setting.[67] Naha *et al*. observed that overexpression of acrB, tolC, ramA, and soxS conferred colistin resistance in a CR *K. pneumoniae* ST147, which could have been the resistance mechanism in the *C. freundii* isolate from Malaysia.[35, 67] Although *mcr* genes, and not chromosomal mutations, are the most common mechanisms mediating polymyxin resistance globally,[1, 2, 4, 9] an *mcr-1* was only identified in one study (in *E. aerogenes*, now *K. aerogenes*) and interestingly, from the vaginal secretions of a pregnant woman.[38]

Hence, we propose the adoption of vaginal swabbing to screen for CR-/PR-GNB in pregnant women besides rectal swabbing as such important pathogens can be missed if they do not occur in the gut. Furthermore, the presence of CR-/PR-GNB in the vagina could explain the colonization of toddlers, infants, neonates, and wards with these MDR pathogens as they can pick up these bacteria during birth.[38, 41] If gut colonization of pregnant women with CR-GNB could potentially lead to colonization and/or infection of the neonate,[22, 24, 68] then vaginal colonization could even lead to a more direct effect than gut colonization, making vaginal screening of pregnant women an important means of preventing CR-/PR-GNB infections and mortalities in neonates. The means by which toddlers, infants, and neonates become colonized by CR-/PR-GNB remains enigmatic, although direct inoculation from mothers is highly likely. Nevertheless, neonatal colonization with CR-/PR-GNB is not only obtained from the mother as observed by other clinicians.[20, 23, 26, 41, 64, 69, 70]

In particular, neonates can become colonized and later infected with CR-/PR-GNB during antibiotic therapy,[23, 71] long stay in wards (NICU) with endemic CR-/PR-GNB,[42, 70] handling by healthcare workers, breastfeeding by colonized mothers, intubation, during labor or cesarean section,[24, 68, 69] mechanized ventilation, from the community,[23, 64] and so on (Tables S1–S4, online only). To establish a direct relationship between mother and infant colonization and with infant colonization and infection, then clonal analyses of isolates would have to be undertaken. As yet, studies showing clonal dissemination of the same GNB strains from mother to neonate as well as neonatal infection by self-colonized strains of the same clonality are wanted (Fig. 2). Despite the larger number of studies reporting on pregnant women colonization, there are relatively few reports of pregnant women infected with CR-/PR-GNB, suggesting that colonization does not necessarily lead to infections in pregnant women or that their stronger and much more developed immune system makes them less susceptible to CR-/PR-GNB infections. Hence, CR-/PR-GNB colonization in pregnant women is more of a risk to neonates than to the women themselves. Nevertheless, infection of pregnant women with CR-/PR-GNB could complicate their pregnancies and put their lives at risk.[45]

The clonality of the species infecting both pregnant women and infants was not the same, suggesting little dissemination between colonized mothers and neonates. Furthermore, there were very few clones of *K. pneumoniae* that were seen in at most three countries, with almost all the reported clones of all the species being local and restricted to single hospitals and wards (Fig. 2; Tables S1–S3, online only). This demonstrates the limited circulation of the same clone even within a community, ward, hospital, or country. *E. coli* ST410, which was predominantly reported in China among neonates, has been shown to be an international clone associated with OXA-181 and NDM-5 carbapenemases dissemination through IncX3 and other plasmids.[72-76] However, in this analysis, this clone was associated with NDM-5 only (Table S1, online only), albeit a recent report from Nigeria found this clone to be

harboring NDM-1.[77] *K. pneumoniae* ST258, an internationally disseminated clone, was only found in a pregnant woman from the United States. Notwithstanding, *K. pneumoniae* ST1878, which was found in pregnant women in Algeria, is less internationally disseminated, compared with ST13 (also from the same country), which has been reported globally to be associated with plasmid-borne carbapenemases.[78, 79]

The carbapenemases found in the CR-GNB isolates (from toddlers, infants, and neonates as well as from pregnant women) mirrored the prevalence of carbapenemases found in clinical, animal, and environmental isolates from the same countries and regions (Fig. 7; and Fig. S7, online only). For instance, IMP and VIM are common in Japan/China and Italy (Europe), respectively, OXA-48 is common in the Mediterranean region, Middle East, and South Africa, while KPC and NDM are globally distributed.[48] This global epidemiology of carbapenemases is reflected in the epidemiology of carbapenemases in toddlers, infants, neonates, and pregnant women (Fig. 7; and Table S4, online only), further corroborating the localized transmission of CR-GNB in neonates. As shown in Figure 7 and Table S4 (online only), the OXA-48–positive GNB isolates in infants/neonates were mostly from Africa (Ghana, Morocco, and South Africa), with a few of them being reported in India, Taiwan, and Turkey. In pregnant women, all the OXA-48 genes were reported from Algeria. Thus, most of the OXA-48–like carbapenemases reported among infants and neonates are basically from Africa, which is a most interesting finding.

Even more interesting is the realization that NDM-positive GNB from pregnant women were all reported from only Madagascar, albeit in toddlers, infants, and neonates, they were mostly found in China and India, where it was first discovered from and remains very endemic.[80, 81] In addition, the higher prevalence of NDM, KPC, and OXA-48–like carbapenemases over VIM and IMP[48] also reflects the global prevalence of these β-lactamases in neonates (Fig. 7). The carbapenemases were mainly found on mobile plasmids (IncFII, IncX$_3$, and IncN), integrons (*IntI*1), ISs (*IS*Kpn7, *IS*Kpn6, *IS*Aba125, and *IS*26), and transposons (Tn*4401b*), which facilitates their movement from chromosomes to plasmids, plasmids to plasmids, and between cells of the same or different species.[13, 20, 26, 31, 32, 35, 36, 41, 44, 63, 82]

Specifically, NDM-5 carbapenemases were found on IncX$_3$ and IncFII plasmids in *K. quasipneumoniae* ST476 (in Nigeria) and *E. coli* ST410 (in China), respectively, which were causing outbreaks in NICUs. The immediate genetic environment of the NDM-5 on these plasmids was exactly the same or similar, suggesting that the same plasmids and/or clones were mediating the spread of NDM-5 within and between species.[31, 44] The higher mobility of these carbapenemases may explain their faster dissemination between neonates in wards and hospitals during outbreaks and evinces the need to undertake molecular analyses of isolates to comprehend their epidemiology.[1, 2, 4, 9, 48]

Not all CR-GNB isolates contained carbapenemases. Isolates without carbapenemases, but with ESBLs, such as CTX-M, TEM, SHV, and OXA, coupled with the loss or downregulation of porins OmpK35 and OmpK36, had very high resistance to carbapenems.[27, 43, 62] In Italy, such strains resulted in mortalities as high as 28.5%.[62]

Prevalence of CR-/PR-GNB and carbapenemases was higher in countries with higher GNB isolation rates (Figs. 1-7), which is expected as most clinical investigations are undertaken in response to increasing infections or outbreaks. Furthermore, susceptible GNB are hardly published except when they are isolated together with resistant GNB, making the literature skewed toward resistant GNB cases. Higher reports of carbapenemases were observed in

China, India, Ghana, Italy, Hungary, Turkey, South Africa, Algeria, and Madagascar (in pregnant women), which does not fully reflect the carbapenem resistance rates shown in Figure 6 as not all studies investigated the underlying carbapenem resistance mechanism. For instance, Cambodia, Egypt, Greece, Pakistan, Taiwan, Thailand, and the United States had higher resistance rates (Fig. S6, online only), but very few or no reports of carbapenemases. Whereas this may be due to financial limitations in low- and middle-income countries (LMICs), the same can not be true of wealthier countries, such as Taiwan, Thailand, and the United States. Hence, there is the need for increasing awareness among clinicians and researchers on the need to undertake advanced genomics analyses of resistant GNB to facilitate epidemiological analyses and interventions.

On the other hand, countries with a higher prevalence of carbapenemases and carbanepem resistance rates should immediately institute effective infection control and prevention practices as sufficient evidence indicates that this can both prevent and contain CR-/PR-GNB outbreaks.[40, 66] Particularly, the nosocomial colonization of neonates with clonally related carbapenemase-producing *P. aeruginosa* in an NICU and the subsequent isolation of a VIM-14 carbapenemase in the same pathogen in an NICU, both in Italy,[83, 84] depict the endemic presence of this carbapenemase-producing pathogen within those periods in the affected regions. This signals the urgency required in instituting effective interventions to clear these MDR pathogens and save further lives as outbreaks in NICUs are associated with very high mortalities.[29, 53, 57] It is notable that the mortality rates shown in Figure S6 (online only) do not reflect the pattern of the nominal figures in Figure 6, which is due to the fact that countries with a higher number of absolute infant deaths (e.g., India) had lower percentage mortalities (4.1%) owing to their higher sample sizes ($n = 1501$).

A very worrying observation is the higher concentration of CR-GNB among infants and higher infant deaths among South Asian countries compared with other parts of the world (Figs. 1-4). On the contrary, a higher number of CR-GNB were reported among pregnant women in Eastern Africa (Kenya and Madagascar), Algeria, and Mexico than in other parts of the world. These statistics show that CR-/PR-GNB are common in LMICs than in developed countries, which most likely will be a reflection of the hygienic and infection prevention and control practices in the hospitals in these countries as well as of the sanitary conditions existing in communities in LMICs.[85-87] The age profiles of the mortalities show that neonates are more susceptible to CR-GNB than pregnant women, making CR-GNB a concerning cause of infant mortalities in LMICs (Fig. 3). Hence, improving hygienic and sanitary practices in both hospitals and communities will most likely help reduce the dissemination of these MDR pathogens.

Common risk factors for getting CR-/PR-GNB infections across all countries have been stated above; they include colonization of the infant or mother with CR-/PR-GNB, long-term hospitalization in NICUs, undergoing invasive procedures and previous, ongoing, or longer antibiotic therapy, mechanical ventilation, preterm state, and low birth weight (Fig. 5).[22, 58, 60] Interestingly, neonatal colonization with CR-/PR-GNB during an NICU stay can later result in community and nosocomial infections, as described by Vergadi *et al*.[64] Sadly, infant mortalities from CR-/PR-GNB remain substantially high in many countries, including China and Brazil (Fig. 6), albeit treatment with carbapenems (at higher doses), colistin, and tigecycline has been shown to be very efficient in clearing the infection and saving lives.[7, 10, 28, 44, 59, 88] Instructively, Kumar *et al*. observed that the absence of effective treatment led to higher all-cause mortalities in neonates.[57]

Conclusion, limitations, and future perspectives

Evidently, carbapenem and polymyxin resistance is common in GNB isolated from toddlers, infants, neonates, and pregnant women, with high resistance rates and diverse carbapenemases globally, particularly in India, China, Pakistan, Thailand, Algeria, South Africa, Turkey, Ghana, Hungary, and Italy. In almost all these countries, there were local clonal outbreaks involving *A. baumanii, E. coli, Enterobacter* spp.*, K. pneumoniae*, and *P. aeruginosa* clones hosting mobile plasmids, integrons, ISs, and transposons on which *bla*NDM, *bla*KPC, *bla*OXA-48, *bla*IMP, *bla*VIM, and *bla*GES-5 carbapenemases were found. Whereas colonization in pregnant women is a risk factor for neonatal infections and outbreaks, infections in pregnant women can complicate their pregnancies. Infection with CR-/PR-GNB has been associated with very high mortality rates among toddlers, infants, and neonates, necessitating urgent infection control interventions to prevent further outbreaks and safeguard lives. The commonality of risk factors for getting CR-/PR-GNB infections in all countries indicates that adopting standard and strict infection control measures in all healthcare centers globally can contain these pathogens from causing further infections. Several outbreaks have been contained with strict infection control interventions.

Fortunately, the treatment of CR-/PR-GNB infections is possible with polymyxins, higher doses of carbapenems, tigecycline, amikacin, and avibactam/sulbactam–cephalosporin/carbapenem combinations. Such treatment protocols and medicines must be distributed to all pediatricians and enforced to reduce infant mortalities. Nevertheless, caution with antibiotic therapy (stewardship) must also be advised, as it is a risk factor for acquired carbapenem resistance colonization or infection.

Going forward, periodic genomic (molecular) surveillance/screening of hospitals (wards) and patients must be instituted to preemptively identify patients with CR-/PR-GNB infections or colonization. Particularly, vaginal and rectal screening of pregnant women may help in surveillance exercises. Further genomic analyses to identify the clones, plasmids, and resistance mechanisms shall inform the appropriate intervention to adopt to break the chain of transmission.

Worryingly, most CR-/PR-GNB cases and deaths were reported in LMICs, which could be attributed to poorer hygienic, sanitary, and infection control and prevention practices in these countries, putting the lives of pregnant women and infants at risk. Owing to the limited laboratory resources in these LMICs, many of the studies did not report on the underlying molecular mechanisms of resistance, which limited the analyses on carbapenemases, *mcr* genes, and associated mobile genetic elements. Furthermore, not all studies typed their isolates, which made it difficult to determine the role of clones in the dissemination of carbapenem and polymyxin resistance among infants, neonates, and pregnant women globally.

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Author contributions

J.O.S. designed, supervised, and undertook the study; searched the literature; and undertook all analyses, image design, and write-up of the manuscript. M.A.R. conducted a systematic literature search/review, selection/screening, quality assessment, data extraction and management in a data registration system, data organization in a meaningful manner, descriptive and count data analysis, generation of frequency charts, and drafting of the literature and database search strategy section of manuscript. P.B.F. reviewed the manuscript.

Competing interests

The authors declare no competing interests.

References

1 Osei Sekyere, J. & M.A. Reta. 2020. Genomic and resistance epidemiology of Gram-negative bacteria in Africa: a systematic review and phylogenomic analyses from a One Health perspective. *mSystems* 5: e00897–20.

2 Kopotsa, K., N.M. Mbelle & J. Osei Sekyere. 2020. Epigenomics, genomics, resistome, mobilome, virulome and evolutionary phylogenomics of carbapenem-resistant *Klebsiella pneumoniae* clinical strains. *Microb. Genomics* 6: mgen000474.

3 Osei Sekyere, J. & E. Mensah. 2020. Molecular epidemiology and mechanisms of antibiotic resistance in *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. in Africa: a systematic review from a One Health perspective. *Ann. N.Y. Acad. Sci.* 1465: 29– 58.

4 Osei Sekyere, J., N.E. Maningi, L. Modipane & N.M. Mbelle. 2020. Emergence of *mcr-9.1* in ESBL-producing clinical *Enterobacteriaceae* in Pretoria, South Africa: global evolutionary phylogenomics, resistome and mobilome. *mSystems* 5: e00148– 20.

5 Van Boeckel, T.P. *et al*. 2019. Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science* 365: eaaw1944.

6 Osei Sekyere, J., U. Govinden, L.A. Bester & S.Y. Essack. 2016. Colistin and tigecycline resistance in carbapenemase-producing Gram negative bacteria: emerging resistance mechanisms and detection methods. *J. Appl. Microbiol.* 121: 601– 617.

7 Cagan, E., E. Kiray Bas & H.S. Asker. 2017. Use of colistin in a neonatal intensive care unit: a cohort study of 65 patients. *Med. Sci. Monit.* 23: 548– 554.

8 Osei Sekyere, J. 2016. Current state of resistance to antibiotics of last-resort in South Africa: a review from a public health perspective. *Front. Public Health* 4: 209.

9 Mmatli, M., N.M. Mbelle, N.E. Maningi & O. Sekyere. 2020. Emerging transcriptional and genomic mechanisms mediating carbapenem and polymyxin resistance in *Enterobacteriaceae*: a systematic review of current reports. *mSystems* 5: e00783–20.

10 Almohammady, M.N., E.M. Eltahlawy & N.M. Reda. 2020. Pattern of bacterial profile and antibiotic susceptibility among neonatal sepsis cases at Cairo University Children Hospital. *J. Taibah Univ. Med. Sci.* 15: 39– 47.

11 Karaaslan, A. *et al*. 2016. Intravenous colistin use for multidrug-resistant Gram-negative infections in pediatric patients. *Balkan Med. J.* 33: 627– 632.

12 Mularoni, A. *et al*. 2019. Epidemiology and successful containment of a carbapenem-resistant Enterobacteriaceae outbreak in a Southern Italian Transplant Institute. *Transpl. Infect. Dis.* 21: e13119.

13 Ahmad, N., S.M. Ali & A.U. Khan. 2019. Molecular characterization of novel sequence type of carbapenem-resistant New Delhi metallo-beta-lactamase-1-producing *Klebsiella pneumoniae* in the neonatal intensive care unit of an Indian hospital. *Int. J. Antimicrob. Agents* 53: 525– 529.

14 Aguilera-Alonso, D., L. Escosa-García, J. Saavedra-Lozano, *et al*. 2020. Carbapenem-resistant Gram-negative bacterial infections in children. *Antimicrob. Agents Chemother.* 64: e02183–19.

15 Tacconelli, E. *et al*. 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.* 18: 318– 327.

16 Asokan, G.V., T. Ramadhan, E. Ahmed & H. Sanad. 2019. WHO Global Priority Pathogens List: a bibliometric analysis of Medline-Pubmed for knowledge mobilization to infection prevention and control practices in Bahrain. *Oman Med. J.* 34: 184– 193.

17 Jajoo, M. *et al*. 2018. Alarming rates of antimicrobial resistance and fungal sepsis in outborn neonates in North India. *PLoS One* 13: e0180705.

18 Essel, V. *et al*. 2020. A multisectoral investigation of a neonatal unit outbreak of *Klebsiella pneumoniae* bacteraemia at a regional hospital in Gauteng Province, South Africa. *South African Med. J.* 110: 783– 790.

19 Thatrimontrichai, A. *et al*. 2016. Risk factors and outcomes of carbapenem-resistant *Acinetobacter baumannii* ventilator-associated pneumonia in the neonate: a case–case–control study. *J. Infect. Chemother.* 22: 444– 449.

20 Ahmad, N., S. Khalid, S.M. Ali & A.U. Khan. 2018. Occurrence of *bla*NDM variants among Enterobacteriaceae from a neonatal intensive care unit in a Northern India Hospital. *Front. Microbiol.* 9: 407.

21 Chabah, M. *et al*. 2016. Healthcare-associated infections due to carbapenemase-producing Enterobacteriaceae: bacteriological profile and risk factors. *Med. Mal. Infect.* 46: 157– 162.

22 Chereau, F. *et al*. 2015. Colonization of extended-spectrum-β-lactamase- and NDM-1-producing Enterobacteriaceae among pregnant women in the community in a low-income country: a potential reservoir for transmission of multiresistant Enterobacteriaceae to neonates. *Antimicrob. Agents Chemother.* 59: 3652– 3655.

23 Clock, S.A. *et al*. 2017. Colonization with antimicrobial-resistant Gram-negative bacilli at neonatal intensive care unit discharge. *J. Pediatr. Infect. Dis. Soc.* 6: 219– 226.

24 Mairi, A. *et al*. 2019. Carbapenemase-producing Enterobacteriaceae among pregnant women and newborns in Algeria: prevalence, molecular characterization, maternal–neonatal transmission, and risk factors for carriage. *Am. J. Infect. Control* 47: 105– 108.

25 Literacka, E. *et al*. 2020. Spread of *Klebsiella pneumoniae* ST45 producing GES-5 carbapenemase or GES-1 extended-spectrum β-lactamase in newborns and infants. *Antimicrob. Agents Chemother.* 64: 6– 9.

26 Arhoune, B. *et al*. 2017. Rectal carriage of extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae among hospitalised neonates in a neonatal intensive care unit in Fez, Morocco. *J. Glob. Antimicrob. Resist*. 8: 90– 96.

27 Datta, S. *et al*. 2014. A five-year experience of carbapenem resistance in Enterobacteriaceae causing neonatal septicaemia: predominance of NDM-1. *PLoS One* 9: e112101.

28 Huang, X. *et al*. 2018. Characteristics of NDM-1-producing *Klebsiella pneumoniae* ST234 and ST1412 isolates spread in a neonatal unit. *BMC Microbiol.* 18: 186.

29 Johnson, J. *et al*. 2020. High burden of bloodstream infections associated with antimicrobial resistance and mortality in the neonatal intensive care unit in Pune, India. *Clin. Infect. Dis.* https://doi-org.uplib.idm.oclc.org/10.1093/cid/ciaa554.

30 Liu, J. *et al*. 2018. Emergence and establishment of KPC-2-producing ST11 *Klebsiella pneumoniae* in a general hospital in Shanghai, China. *Eur. J. Clin. Microbiol. Infect. Dis.* 37: 293– 299.

31 Brinkac, L.M. *et al*. 2019. Emergence of New Delhi metallo-beta-lactamase (NDM-5) in *Klebsiella quasipneumoniae* from neonates in a Nigerian hospital. *mSphere* 4: e00685–18.

32 Islam, M.A. *et al*. 2013. Occurrence and characterization of multidrug-resistant New Delhi metallo-beta-lactamase-1-producing bacteria isolated between 2003 and 2010 in Bangladesh. *J. Med. Microbiol.* 62: 62– 68.

33 Zhu, Y.-Q. *et al*. 2016. Identification of an NDM-5-producing *Escherichia coli* sequence type 167 in a neonatal patient in China. *Sci. Rep.* 6: 29934.

34 Yap, P.S.X. *et al*. 2016. Intestinal carriage of multidrug-resistant gram-negative bacteria in preterm-infants during hospitalization in neonatal intensive care unit (NICU). *Pathog. Glob. Health* 110: 238– 246.

35 Naha, S. *et al*. 2020. KPC-2-producing *Klebsiella pneumoniae* ST147 in a neonatal unit: clonal isolates with differences in colistin susceptibility attributed to AcrAB-TolC pump. *Int. J. Antimicrob. Agents* 55: 105903.

36 Zhao, W., G. Chen, R. Ito, *et al*. 2012. Identification of a plasmid-borne bla IMP-11 gene in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *J. Med. Microbiol.* 61(Pt 2): 246– 251.

37 Ding, Y., Y. Wang, Y. Hsia, *et al*. 2019. Systematic review of carbapenem-resistant Enterobacteriaceae causing neonatal sepsis in China. *Ann. Clin. Microbiol. Antimicrob.* 18: 36.

38 Zeng, K.-J., Y. Doi, S. Patil, *et al*. 2016. Emergence of the plasmid-mediated *mcr*-1 gene in colistin-resistant *Enterobacter aerogenes* and *Enterobacter cloacae*. *Antimicrob. Agents Chemother.* 60: 3862– 3863.

39 Zheng, R. *et al*. 2016. Outbreak of plasmid-mediated NDM-1-producing *Klebsiella pneumoniae* ST105 among neonatal patients in Yunnan, China. *Ann. Clin. Microbiol. Antimicrob.* 15: 10.

40 Yu, J. *et al*. 2016. Nosocomial outbreak of KPC-2- and NDM-1-producing *Klebsiella pneumoniae* in a neonatal ward: a retrospective study. *BMC Infect. Dis.* 16: 563.

41 Roy, S., A.K. Singh, R. Viswanathan, *et al*. 2011. Transmission of imipenem resistance determinants during the course of an outbreak of NDM-1 *Escherichia coli* in a sick newborn care unit. *J. Antimicrob. Chemother.* 66: 2773– 2780.

42 Poirel, L. *et al*. 2014. Spread of NDM-1-producing *Enterobacteriaceae* in a neonatal intensive care unit, Istanbul, Turkey. *Antimicrob. Agents Chemother.* 58: 2929– 2933.

43 Jin, Y. *et al*. 2015. Outbreak of multidrug resistant NDM-1-producing *Klebsiella pneumoniae* from a neonatal unit in Shandong Province, China. *PLoS One* 10: e0119571.

44 Li, J. *et al*. 2020. Emergence of an NDM-5-producing *Escherichia coli* sequence type 410 clone in infants in a children's hospital in China. *Infect. Drug Resist.* 13: 703– 710.

45 Khatri, A. *et al*. 2015. Community-acquired pyelonephritis in pregnancy caused by KPC-producing *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 59: 4375– 4378.

46 Macharashvili, N. *et al*. 2009. Etiology of neonatal blood stream infections in Tbilisi, Republic of Georgia. *Int. J. Infect. Dis.* 13: 499– 505.

47 Mairi, A., A. Pantel, A. Sotto, *et al*. 2018. OXA-48-like carbapenemases producing Enterobacteriaceae in different niches. *Eur. J. Clin. Microbiol. Infect. Dis.* 37: 587– 604.

48 Kopotsa, K., J. Osei Sekyere & N.M. Mbelle. 2019. Plasmid evolution in carbapenemase-producing Enterobacteriaceae: a review. *Ann. N.Y. Acad. Sci.* 1457: 61– 91.

49 Mbelle, N. *et al*. 2020. Genomic analysis of two drug-resistant clinical *Morganella morganii* strains isolated from UTI patients in Pretoria, South Africa. *Lett. Appl. Microbiol.* 70: 21– 28.

50 Mbelle, N.M. *et al*. 2019. Genomic analysis of a multidrug-resistant clinical *Providencia rettgeri* (PR002) strain with the novel integron ln1483 and an A/C plasmid replicon. *Ann. N.Y. Acad. Sci.* 1462: 92– 103.

51 Osei Sekyere, J. & M.A. Reta. 2020. Global evolutionary epidemiology, phylogeography and resistome dynamics of *Citrobacter species*, *Enterobacter hormaechei*, *Klebsiella variicola*, and *Proteeae clones*: a One Health analyses. *Environ. Microbiol.* https://doi-org.uplib.idm.oclc.org/10.1101/2020.05.21.20109504.

52 Stoesser, N. *et al*. 2014. Genome sequencing of an extended series of NDM-producing *Klebsiella pneumoniae* isolates from neonatal infections in a Nepali hospital characterizes the extent of community- versus hospital-associated transmission in an endemic setting. *Antimicrob. Agents Chemother.* 58: 7347– 7357.

53 Saleem, A.F., F.N. Qamar, H. Shahzad, *et al*. 2013. Trends in antibiotic susceptibility and incidence of late-onset *Klebsiella pneumoniae* neonatal sepsis over a six-year period in a neonatal intensive care unit in Karachi, Pakistan. *Int. J. Infect. Dis.* 17: e961– e965.

54 Sultan, A.M. & W.A. Seliem. 2018. Identifying risk factors for healthcare-associated infections caused by carbapenem-resistant *Acinetobacter baumannii* in a neonatal intensive care unit. *Sultan Qaboos Univ. Med. J.* 18: e75– e80.

55 Indhar, F., M.A. Durrani, A. Bux & M. Sohail. 2017. Carbapenemases among *Acinetobacter species* isolated from NICU of a tertairy care hospital in Karachi. *J. Pak. Med. Assoc*. 67: 1547– 1551.

56 Al-lawama, M., H. Aljbour, A. Tanash & E. Badran. 2016. Intravenous colistin in the treatment of multidrug-resistant *Acinetobacter* in neonates. *Ann. Clin. Microbiol. Antimicrob.* 15: 1– 4.

57 Kumar, A., V.S. Randhawa, N. Nirupam, *et al*. 2014. Risk factors for carbapenem-resistant *Acinetobacter baumanii* blood stream infections in a neonatal intensive care unit, Delhi, India. *J. Infect. Dev. Ctries.* 8: 1049– 1054.

58 Lee, H.Y. *et al*. 2018. Risk factors and molecular epidemiology of *Acinetobacter baumannii* bacteremia in neonates. *J. Microbiol. Immunol. Infect.* 51: 367– 376.

59 Tekin, R., T. Dal, H. Pirinccioglu & S.E. Oygucu. 2013. A 4-year surveillance of device-associated nosocomial infections in a neonatal intensive care unit. *Pediatr. Neonatol.* 54: 303– 308.

60 Li, P. *et al*. 2017. ST37 *Klebsiella pneumoniae*: development of carbapenem resistance *in vivo* during antimicrobial therapy in neonates. *Future Microbiol*. 12: 891– 904.

61 Chen, D. *et al*. 2019. Co-outbreak of multidrug resistance and a novel ST3006 *Klebsiella pneumoniae* in a neonatal intensive care unit: a retrospective study. *Medicine (Baltimore)* 98: e14285.

62 Arena, F. *et al*. 2013. Large oligoclonal outbreak due to *Klebsiella pneumoniae* ST14 and ST26 producing the FOX-7 AmpC β-lactamase in a neonatal intensive care unit. *J. Clin. Microbiol.* 51: 4067– 4072.

63 Yu, J. *et al*. 2017. Outbreak of nosocomial NDM-1-producing *Klebsiella pneumoniae* ST1419 in a neonatal unit. *J. Glob. Antimicrob. Resist.* 8: 135– 139.

64 Vergadi, E., M. Bitsori, S. Maraki & E. Galanakis. 2017. Community-onset carbapenem-resistant *Klebsiella pneumoniae* urinary tract infections in infancy following NICU hospitalisation. *J. Pediatr. Urol.* 13: 495.e1– 495.e6.

65 Liu, Y. *et al*. 2013. Acquisition of carbapenem resistance in multiresistant *Klebsiella pneumoniae* isolates of sequence type 11 at a university hospital in China. *Diagn. Microbiol. Infect. Dis.* 76: 241– 243.

66 Kong, Z. *et al*. 2019. First reported nosocomial outbreak of NDM-5-producing *Klebsiella pneumoniae* in a neonatal unit in China. *Infect. Drug Resist.* 12: 3557– 3566.

67 Yap, P.S.X., A. Ahmad Kamar, C.W. Chong, *et al*. 2020. Whole genome analysis of multidrug resistant *Citrobacter freundii* B9-C2 isolated from preterm neonate's stool in the first week. *J. Glob. Antimicrob. Resist.* 21: 246– 251.

68 Rawstron, S.A. *et al*. 2018. Perirectal screening for carbapenem-resistant enterobacteriaceae obtained from 100 consecutive healthy pregnant women in labor at a Brooklyn hospital: results and risk factors. *Infect. Control Hosp. Epidemiol.* 39: 369– 371.

69 Kothari, C. *et al*. 2013. Community acquisition of beta-lactamase producing Enterobacteriaceae in neonatal gut. *BMC Microbiol.* 13: 136.

70 Turner, P. *et al*. 2016. High prevalence of antimicrobial-resistant Gram-negative colonization in hospitalized Cambodian infants. *Pediatr. Infect. Dis. J.* 35: 856– 861.

71 Zhu, J. *et al*. 2015. *Klebsiella pneumoniae*: development of carbapenem resistance due to acquisition of blaNDM-1 during antimicrobial therapy in twin infants with pneumonia. *Front. Microbiol.* 6: 1– 5.

72 Patino-Navarrete, R. *et al*. 2020. Stepwise evolution and convergent recombination underlie the global dissemination of carbapenemase-producing *Escherichia coli*. *Genome Med*. 12: 10.

73 Roer, L. *et al*. 2018. *Escherichia coli* sequence type 410 is causing new international high-risk clones. *mSphere* 3: e00337– 18.

74 Feng, Y. *et al*. 2019. Key evolutionary events in the emergence of a globally disseminated, carbapenem resistant clone in the *Escherichia coli* ST410 lineage. *Commun. Biol.* 2: 1– 13.

75 Abd El Ghany, M. *et al*. 2018. Genomic characterization of NDM-1 and 5, and OXA-181 carbapenemases in uropathogenic *Escherichia coli* isolates from Riyadh, Saudi Arabia. *PLoS One* 13: e0201613.

76 Mbelle, N.M. *et al*. 2019. The resistome, mobilome, virulome and phylogenomics of multidrug-resistant *Escherichia coli* clinical isolates from Pretoria, South Africa. *Sci. Rep.* 9: 1– 43.

77 Olalekan, A. *et al*. 2020. High proportion of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* among extended-spectrum β-lactamase-producers in Nigerian hospitals. *J. Glob. Antimicrob. Resist.* 21: 8– 12.

78 Aires-De-Sousa, M. *et al*. 2019. Epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in a hospital, Portugal. *Emerg. Infect. Dis.* 25: 1632– 1638.

79 Marcade, G. *et al*. 2013. The emergence of multidrug-resistant *Klebsiella pneumoniae* of international clones ST13, ST16, ST35, ST48 and ST101 in a teaching hospital in the Paris region. *Epidemiol. Infect.* 141: 1705– 1712.

80 van Duin, D. & Y. Doi. 2017. The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence* 8: 460– 469.

81 Kumarasamy, K.K. *et al*. 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect. Dis.* 10: 597.

82 Xu, J., W. Lin, Y. Chen & F. He. 2020. Characterization of an IMP-4-producing *Klebsiella pneumoniae* ST1873 strain recovered from an infant with a bloodstream infection in China. *Infect. Drug Resist.* 13: 773– 779.

83 Mammina, C. *et al*. 2008. Nosocomial colonization due to imipenem-resistant *Pseudomonas aeruginosa* epidemiologically linked to breast milk feeding in a neonatal intensive care unit. *Acta Pharmacol. Sin.* 29: 1486– 1492.

84 Mazzariol, A. *et al*. 2011. A novel VIM-type metallo-beta-lactamase (VIM-14) in a *Pseudomonas aeruginosa* clinical isolate from a neonatal intensive care unit. *Clin. Microbiol. Infect.* 17: 722– 724.

85 Tran, D.M. *et al*. 2019. High prevalence of colonisation with carbapenem-resistant Enterobacteriaceae among patients admitted to Vietnamese hospitals: risk factors and burden of disease. *J. Infect.* 79: 115– 122.

86 Nadimpalli, M.L. *et al*. 2020. Urban informal settlements as hotspots of antimicrobial resistance and the need to curb environmental transmission. *Nat. Microbiol.* 5: 787– 795.

87 Møller Aarestrup, F., M.E.J. Woolhouse, F.M. Aarestrup & M.E.J. Woolhouse. 2020. Using sewage for surveillance of antimicrobial resistance. *Science* 367: 630– 632.

88 Choudhry, S., E. Ahmad, A. Batool & N. Raja. 2017. Use of colistin for the treatment of multi drug resistant isolates in neonates. *J. Pak. Med. Assoc.* 67: 1157– 1160.