Impact of Intrapartum Azithromycin on the Carriage and Antibiotic Resistance of *Escherichia coli* and *Klebsiella pneumoniae* in Mothers and their Newborns: a sub-study of a Randomized Double-Blind Trial Conducted in The Gambia and Burkina Faso

Pauline Getanda¹, Isatou Jagne¹, Joel D. Bognini², Bakary Sanyang¹, Saffiatou Darboe¹, Ellen Sambou¹, Momodou Barry¹, Kady Kassibo¹, Aminata Cham¹, Harriet Mendy¹, Bintou K. J. Singateh¹, Ebrahim Ndure¹, Toussaint Rouamba², Abdoulaye Bojang¹, Christian Bottomley³, Benjamin P. Howden⁴, Umberto D’Alessandro¹, Halidou Tinto², Anna Roca¹ and PregAnZI-2 carriage study group¹,²*.

¹Disease Control and Elimination Theme, Medical Research Council Unit, The Gambia at London School of Hygiene and Tropical Medicine (MRCG @ LSHTM), Banjul, The Gambia; ²Institut de Recherche en Sciences de la Santé, Clinical Research Unit of Nanoro (CRUN), Nanoro, Burkina Faso.; ³ Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom; ⁴Microbiological Diagnostic Unit (MDU) Public Health Laboratory, Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia.

**Background:** Limited data exists on effects of intrapartum azithromycin on prevalence of carriage and antibiotic resistance of Enterobacterales.

*Study group team members are listed in the acknowledgements.

**Corresponding author:** Anna Roca, MRC Unit The Gambia at LSHTM. Email: aroca@mrc.gm. Tel: +34674986213 MRC Unit The Gambia at LSHTM, Atlantic Boulevard, Fajara P.O. Box 273, Banjul, The Gambia.

**Alternate corresponding author:** Pauline Getanda, MRC Unit The Gambia at LSHTM. Email: Pauline.Getanda@lshtm.ac.uk. Tel: +61415187103 MRC Unit The Gambia at LSHTM Atlantic Boulevard, Fajara P.O. Box 273, Banjul, The Gambia.

© The Author(s) 2024. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
Methods: We conducted a randomized trial in Gambia and Burkina Faso where women received intrapartum azithromycin (2g) or placebo. We determined impact of treatment on prevalence of carriage and antibiotic resistance of *Escherichia coli* and *Klebsiella pneumoniae* by analysing rectal swabs (RS), nasopharyngeal swabs (NPS), breast milk and recto-vaginal swabs (RVS). Bacteria were isolated microbiologically; antibiotic susceptibility was confirmed with an E-test. Prevalence ratios (PR) with 95% confidence intervals (CI’s) were used for comparison between arms.

Results: In infants, *E. coli* carriage in RS was lower in the intervention than placebo arm at days 6 (63.0% vs. 75.2%, PR, 0.84; CI, 0.75-0.95) and 28 (52.7% vs. 70.4%, 0.75; 0.64-0.87) post-intervention. Prevalence of azithromycin-resistant *E. coli* was higher in the azithromycin arm at days 6 (13.4% vs. 3.6%, 3.75; 1.83-7.69) and 28 (16.4% vs. 9.6%, 1.71; 1.05-2.79). For *K. pneumoniae*, carriage in RS was higher in the intervention than placebo arm at days 6 (49.6% vs. 37.2%, 1.33; 1.08-1.64) and 28 (53.6% vs. 32.9%, 1.63; 1.31-2.03). Prevalence of azithromycin-resistant *K. pneumoniae* was higher in the azithromycin arm at day 28 (7.3% vs. 2.1%, 3.49; 1.30-9.37). No differences were observed for other sample types.

Conclusion: Intrapartum azithromycin decreased *E. coli* carriage but increased both *K. pneumoniae* carriage and azithromycin resistance in both bacteria. These data need to be considered together with efficacy results to balance the potential short- and long-term impact of the intervention.

Clinical Trials registration: www.clinicaltrials.gov: NCT03199547

Keywords: Intrapartum azithromycin, *Escherichia coli*, *Klebsiella pneumoniae*, bacterial carriage, antibiotic resistance.

INTRODUCTION

Efforts to reduce global neonatal mortality rates have led to a 50% decrease, from 36.6 to 17.5 per 1000 live births between 1990 and 2019 [1]. Nevertheless, progress varies across regions [2]. Over the same period, neonatal mortality rates in sub-Saharan Africa decreased by 26%, currently representing 43% of global neonatal deaths [1]. Neonatal sepsis, a major contributor to neonatal mortality [3], is often caused by *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp., with varying prevalence in different African sub-regions [4, 5]. For example, *S. aureus* sepsis is more prevalent in West Africa, while *Klebsiella* spp. sepsis is more common in Central and South Africa [4]. Maternal vaginal *S. aureus* colonization, which is correlated with neonatal colonization and subsequent disease, is estimated to be 16% in West Africa 29%, in Central Africa and 2-8% in East Africa [6-9].

Azithromycin, a second-generation macrolide antibiotic, exhibits broad-spectrum activity against gram-positive and some gram-negative bacteria [10]. Its oral administration results in rapid
absorption reaching peak concentrations in blood or tissues within 2-3 hours [11], becoming a
potential prophylactic antibiotic for preventing neonatal and maternal infections.

Recent double-blinded randomized trials, PregAnZI-2 and A-PLUS, explored the use of
azithromycin to decrease neonatal sepsis and mortality across nine African and Asian countries
[12, 13]. Although no reduction in neonatal sepsis and mortality was observed, a significant
impact in reducing maternal infections [12], including puerperal sepsis [13], was noted. The
PregAnZI-2 trial, conducted in West Africa, also reported a reduction in neonatal infections and
a lower rate of prescribed antibiotics during the neonatal period [12].

Our earlier research showed that this intervention reduces gram-positive bacterial colonization in
mothers and newborns throughout the neonatal period, including S. aureus [6]. Despite this, a
temporary increase in azithromycin-resistant S. aureus lasting between 1-12 months was
observed [14]. Additionally, intrapartum azithromycin lowered carriage of Streptococcus
pneumoniae and Groups A and B Streptococcus, without increasing antibiotic resistance [6, 15].
Data on the effect of intrapartum azithromycin on carriage and antibiotic resistance of gram-
negative bacteria causing neonatal sepsis, E. coli and K. pneumoniae, are scarce [16]. It is
important to evaluate the impact of the intervention on these two gram-negative bacteria due to
their role in neonatal sepsis and their rising rates of multidrug resistance which severely limits
treatment options [17]. The study presented here aims to determine the effect of intrapartum
azithromycin on the prevalence of carriage and antibiotic resistance of E. coli and K. pneumoniae
among mother-infant pairs from The Gambia and Burkina Faso.

METHODS

Trial overall design

PregAnZI-2 was a phase III double-blind placebo-controlled randomized clinical trial that
recruited 12,000 women in The Gambia and Burkina Faso to receive either oral azithromycin
(2g) or placebo during labor (ratio 1:1). Women, 16+ years were consented during ante-natal
visits and enrolled in the trial after oral consent at study health facilities during labor [18].
(www.clinicaltrials.gov_NCT03199547).

Study sites

In The Gambia, women were recruited from two peri-urban government health facilities located
close to the capital, Banjul. In Burkina Faso, women were recruited in eight health facilities in
rural central districts of Nanoro and Yako (Supplementary Figure 1).
The carriage sub-study

A sub-group of 250 mother/infant pairs per country participated in this sub-study. They were enrolled into the trial between 23rd January 2019 and 27th March 2020 in The Gambia, and between 02nd April 2019 and 08th April 2020 in Burkina Faso.

Biological samples were collected pre-intervention until 4 months post-intervention. A maternal nasopharyngeal (NPS) and rectovaginal swab (RVS) were collected during labor before the intervention. Within 4 hours after birth, an NPS and a Rectal Swab (RS) were collected from newborns. Additional samples were collected during household visits: from mothers; NPS at day-6, breast milk (BM) at day-6, 28 and month-4 and from infants; NPS and RS at day-6, 28 and month-4. For The Gambia, the last two sample collection timepoints were affected by the state of emergency declared in March 2020 due to the COVID-19 pandemic [19].

Sample handling and laboratory methods

RVS were collected using a sterile cotton swab inserted 2-3 cm into the vagina, rotated in circular motion for 5 seconds. The same swab was inserted 2-3 cm through the anal sphincter, rotated in circular motion for 5 seconds. The latter procedure was done to collect RS from infants. Sample collection for NPS and BM samples was done as previously described [20]. Swabs were placed in a vial containing skim milk-tryptone-glucose-glycerol transport medium in a cold box and transported to the labs within 8 hours. On arrival samples were vortexed for 20 seconds and stored at -70°C for batch processing. Samples collected in Burkina Faso were shipped to The Gambia on dry ice and stored as described above.

Identification of E. Coli and K. Pneumoniae

E. coli and K. pneumoniae were isolated from mothers’ BM and RVS and newborns’ RS. In addition, K. pneumoniae was isolated from participants’ NPS (Supplementary Figure 2). Samples thawed on ice, were vortexed briefly and an aliquot of 50μl dispensed onto MacConkey agar (Oxoid, UK) was streaked for selective isolation of E. coli and K. pneumoniae as previously described [16]. For E. coli, identification was done for each morphologically distinct suspected colony when more than one was available and each stored separately.

Antibiotic susceptibility

We performed disc diffusion on 3-5 well-isolated E. coli colonies or K. pneumoniae as previously described [16]. We tested for susceptibility to azithromycin and nine other antibiotics (ampicillin, trimethoprim-sulfamethoxazole, gentamicin, ciprofloxacin, cefoxitin, ceftazidime, cefotaxime, amoxicillin-clavulanic acid, and meropenem). Production of Extended Spectrum β-Lactamase (ESBL) was determined using double disc-synergy diffusion test (CLSI) [21]. The Minimum Inhibitory Concentration (MIC) for all azithromycin non-susceptible isolates, 5% of azithromycin susceptible isolates, all ESBL producers and 2% of ESBL non-producers was
determined using E-test strips (Biomérieux, Marcy l’Etoile, France) per manufacturer’s instructions. Antibiotic concentrations are included in prevalence tables. CLSI lacks clinical breakpoints for *E. coli* and *K. pneumoniae* azithromycin resistance, details of cutoffs are in Table 2/3 [22, 23]. The strains, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as controls.

**Statistical analysis**

The prevalence of bacterial carriage and antibiotic resistance was compared between trial arms using PR, with corresponding 95% CI’s. Fisher’s exact test was used to obtain p-values, p<0.05 was considered significant. Stata version 18 was used for all analyses. In the main analyses, the total number of available samples was used as denominator. When we identified >1 *E. coli* isolate per RVS and RS, to calculate prevalence of *E. coli* carriage at a particular site we considered a participant a “carrier” if at least one *E. coli* was isolated from the sample, and a sample was considered “resistant” for a specific antibiotic if at least one resistant *E. coli* isolate was present. For *K. pneumoniae*, only one isolate per sample was identified and tested for resistance. In addition, we determined the frequency of antibiotic resistance in infants’ RS for samples that were positive for *E. coli* and *K. pneumoniae*.

**Ethical considerations**

The trial was approved by the joint Gambia Government/Medical Research Council Unit The Gambia (MRCG) Ethics committee, the Comité d’Ethique pour la Recherche en Santé (CERS) of Burkina Faso and the LSHTM Ethics Committee. Consent was sought concurrently for both the main trial and carriage sub-study when pregnant women attended antenatal clinics.

**RESULTS**

Overall, 500 mother-infant pairs participated in this sub-study, 250 from The Gambia, 250 from Burkina Faso (122 in azithromycin and 128 in placebo arm per country). The proportion of samples collected was >98% at day-0 and 6, 92% at day-28, and 79% at month-4. Details of samples available at each timepoint are in Figure 1 (Trial profile). Baseline characteristics of study arms are shown in Table 1.

**Prevalence of carriage and azithromycin resistance of *E. Coli***

**Study women.** For pre-intervention RVS, prevalence of *E. coli* carriage was similar in azithromycin and placebo arms (68.9% and 67.6% respectively) (Table 2). Prevalence of carriage of azithromycin-resistant isolates was low and ranged between 2.7% and 4.5% (Table 2). For post-intervention samples, there were no differences between arms in the prevalence of carriage of *E. coli* or azithromycin-resistant *E. coli* in BM at any timepoint (Table 2). Analyses stratified by country are in Supplementary Tables 1a/1b.
Study infants. Prevalence of E. coli carriage in infants’ RS samples was lower in azithromycin arm compared to placebo at days 6 (63.0% vs. 75.2%, PR, 0.84; CI, 0.75-0.95, p=0.006) and 28 (52.7% vs. 70.4%, 0.75; 0.64-0.87, p=0.001) (Table 2; Figure 2). Prevalence of azithromycin resistant E. coli in the azithromycin arm was significantly higher at days 6 (13.4% vs. 3.6%, 3.75; 1.83-7.69, p=0.001) and 28 (16.4% vs. 9.6%, 1.71; 1.05-2.79, p=0.036) (Table 2; Figure 2). The frequency of azithromycin resistance among E. coli isolated from RS was higher in the azithromycin arm at day-6 and 28 (Supplementary Table 2). Analyses stratified by country are in Supplementary Tables 1a/1b.

Prevalence of E. Coli resistance to other antibiotics

Study women. For pre-intervention RVS and post-intervention BM, there were no differences between arms in the prevalence of E. coli resistant to ampicillin, trimethoprim-sulfamethoxazole, gentamicin, and ciprofloxacin. For pre-intervention RVS, there was higher prevalence of ESBL carriage (2.0% vs. 0% p=0.027) in azithromycin arm compared to placebo (Supplementary Tables 3a/3b/3c). There was no resistance to meropenem and cefoxitin resistance was low.

Study infants. For infants’ RS, prevalence of carriage of E. coli resistant to ampicillin at days 6 (46.2% vs. 58.4%, 0.80; 0.67-0.94, p=0.009) and 28 (44.1% vs. 59.6%, 0.74; 0.62-0.89, p=0.001) was lower in the azithromycin arm (Supplementary Table 3a; Supplementary Figure 3). Prevalence of carriage of E. coli resistant to trimethoprim-sulfamethoxazole was lower in the azithromycin arm at days 6 (45.4% vs. 57.6%, 0.79; 0.66-0.94, p=0.009) and 28 (42.3% vs. 57.1%, 0.74; 0.61-0.89, p=0.002) (Supplementary Table 3a; Supplementary Figure 3). Prevalence of carriage of E. coli resistant to cefoxitin was lower at day-28 (0% vs. 2.9%, p=0.016) in the azithromycin arm (Supplementary Table 3c; Supplementary Figure 3). Prevalence of E. coli resistant to gentamicin, ciprofloxacin and ESBL carriage was similar between arms (Supplementary Tables 3b/3c; Supplementary Figure 3). No meropenem-resistant E. coli was detected. Details of frequency of antibiotic resistance among E. coli isolated from RS are in Supplementary Table 2.

Prevalence of carriage and azithromycin resistance of K. Pneumoniae

Study women. Prevalence of K. pneumoniae carriage was similar between arms for all samples and timepoints (Table 3). Prevalence of azithromycin-resistant isolates before and after the intervention was low and similar between arms (0.4% vs. 1.6%) (Table 3). Analyses stratified by country are in Supplementary Tables 4a/4b.

Study infants. Prevalence of K. pneumoniae carriage in RS was higher in the azithromycin arm at days 6 (49.6% vs. 37.2%, 1.33; 1.08-1.64, p=0.006) and 28 (53.6% vs. 32.9%, 1.63; 1.31-2.03, p<0.001) (Table 3; Figure 2). For azithromycin-resistant K. pneumoniae in RS, study arms were different at day-28 (7.3% vs. 2.1%, 3.49; 1.30-9.37, p=0.012) in azithromycin arm vs. placebo (Table 3; Figure 2). Details of frequency of azithromycin resistance among K. pneumoniae isolated from RS are in Supplementary Table 5. For NPS, no differences between arms were
found for prevalence of *K. pneumoniae* carriage nor azithromycin resistance (Table 3). Analyses stratified by country are in Supplementary Tables 4a and 4b.

**Prevalence of *K. Pneumoniae* resistance to other antibiotics**

**Study women.** For pre-intervention maternal RVS and post-intervention BM there were no differences between arms in the prevalence of *K. pneumoniae* resistant to trimethoprim-sulfamethoxazole, gentamicin, ciprofloxacin, and ESBL carriage. For pre-intervention maternal RVS, prevalence of *K. pneumoniae* resistant to cefoxitin was higher in the azithromycin arm (2.0% vs. 0%, *p*=0.027) (Supplementary Table 6c). No resistance to meropenem was detected. For maternal NPS, resistance to all antibiotics was either absent or low.

**Study infants.** In RS, resistance to trimethoprim-sulfamethoxazole (23.2% vs. 8.8%, 2.65; 1.65-4.26, *p*<0.001), gentamicin (10.5% vs. 5.0%, 2.09; 1.07-4.10, *p*=0.034), ciprofloxacin (15.5% vs. 5.8%, 2.65; 1.46-4.80, *p*=0.001), and ESBL carriage (9.5% vs. 3.3%, 2.86; 1.30-6.33, *p*=0.007) at day-28 was higher in the azithromycin arm (Supplementary Tables 6a/6b/6c; Supplementary Figure 3); resistance to cefoxitin was low (Supplementary Table 6c) with no resistance to meropenem. The frequency of antibiotic resistance among *K. pneumoniae* isolated from RS samples are in Supplementary Table 5. In NPS, resistance to all tested antibiotics was either low or absent.

**DISCUSSION**

Clinical trials have shown that prophylactic intrapartum azithromycin decreases maternal and neonatal infections [12, 13]. It is important, therefore, to evaluate the effect of this intervention on bacterial colonization and antimicrobial resistance. Previous studies showed the intervention decreases carriage of the main gram-positive bacteria causing sepsis in mothers and newborns, with very little effect on azithromycin resistance [6, 15]. In this study, azithromycin reduced *E. coli* carriage and increased *K. pneumoniae* carriage, predominantly in infants’ RS. The intervention increased the carriage of azithromycin-resistant isolates for both bacteria. It simultaneously decreased the carriage of *E. coli* resistant to other antibiotics and increased the carriage of *K. pneumoniae* resistant to other antibiotics.

In a previous trial conducted in The Gambia following the same design, azithromycin (2g), remained in the maternal breast milk for at least four weeks post-intervention, reaching peak levels on day-6 [24]. The substantial concentration of azithromycin transferred to infants, coupled with the impact on maternal carriage, likely explains the effects observed in infants in this study. However, such an effect on RS carriage of *E. coli* only lasted the neonatal period. These findings are consistent with the effect of azithromycin mass drug administration that reduced the short-term risk of diarrhea in infants aged 2-59 months; diarrheagenic *E. coli* being a major cause of diarrhea at this age [25-27]. *In vitro* experiments have also shown azithromycin is
efficacious against certain strains of pathogenic *E. coli* [28]. Moreover, azithromycin can effectively reduce bacterial shedding in patients with shiga-toxin-producing enteroinvasive *E. coli* (STEC) and travellers’ diarrhoea caused by enterotoxigenic *E. coli* (ETEC) [29, 30]. The increased azithromycin resistance was not matched by an increased *E. coli* resistance to other antibiotics. On the contrary, infants whose mothers had taken intrapartum azithromycin had a lower prevalence of ampicillin, trimethoprim-sulfamethoxazole, and cefoxitin-resistant *E. coli* isolates. There are two plausible explanations; the lower use of prescribed antibiotics in infants from the azithromycin arm due to lower rates of infections observed during the trial [12] may have resulted in a lower selective pressure and thus lower resistance to common antibiotics. Ampicillin and trimethoprim-sulfamethoxazole are broad-spectrum antibiotics often used for the treatment of respiratory, gastrointestinal and urinary tract infections in West Africa [34, 35]. Secondly, lower overall prevalence of *E. coli* carriage in RS would translate to a lower prevalence of carriage of isolates resistant to other antibiotics. The similar frequency of *E. coli* isolates resistant to the different antibiotics in both arms would support this last hypothesis. This decreased prevalence of carriage of *E. coli* resistant isolates is an encouraging result that needs to be interpreted considering further evaluation of the overall effects on the microbiome and resistance to the intervention.

We previously showed a higher carriage of *K. pneumoniae* isolated in BM samples collected after azithromycin treatment [16]. In this study, intrapartum azithromycin increased the risk of *K. pneumoniae* carriage in infants’ RS. The strong effect of azithromycin on gram-positive bacteria [6, 15] and certain gram-negative bacteria as observed with *E. coli* here, may have advantaged *K. pneumoniae* at these body sites. Overgrowth of certain bacterial species after using broad-spectrum antibiotics has been reported. A study investigating the effect of early-life antibiotics on the developing infant gut showed that antibiotic-treated infants had a higher abundance of *Klebsiella* spp. [36]. Nevertheless, this higher *K. pneumoniae* carriage did not increase the incidence of *K. pneumoniae* sepsis in our PregAnZI-2 trial [12] or the A-PLUS trial (conducted in 7 low- and middle-income countries) [13].

In our study, it is possible that the higher prevalence of *K. pneumoniae* carriage resulted in a high carriage of azithromycin-resistant strains in RS, as we previously showed for BM [16]. Indeed, the time of the highest carriage of *K. pneumoniae* (day-28), coincides with that of the highest prevalence of azithromycin-resistant *K. pneumoniae* isolates in infants’ RS. In addition, day-28 was also the timepoint with higher resistance to other tested antibiotics, possibly caused by the same phenomenon. In support of this, we observed a similar trend in the frequency of resistant
isolates for all antibiotics, including azithromycin. The production of ESBL in Enterobacterales mediates simultaneous acquisition of resistance to other classes of antibiotics because resistance genes may be located on the same mobile genetic elements [37] and could have contributed to the increased resistance to other antibiotics in K. pneumoniae at day-28.

This study had some limitations. Although we have shown the effect of the intervention on resistance to azithromycin and other antibiotics in E. coli and K. pneumoniae, we could not ascertain the mechanisms of resistance involved. This requires genomic evaluation to complement phenotypic observations. Also, despite the reduction of E. coli carriage following intrapartum azithromycin, it was not possible to determine whether such reduction is beneficial as we have not distinguished between pathogenic and non-pathogenic E. coli.

CONCLUSION

Intrapartum azithromycin decreases carriage of E. coli and increases carriage of K. pneumoniae in the gut of neonates. The intervention also increases carriage of azithromycin-resistant E. coli and K. pneumoniae isolates, a potential threat to the spread of such resistance to the community. Conversely, this intervention may decrease resistance to other commonly used antibiotics such as ampicillin or trimethoprim-sulfamethoxazole in E. coli either because it decreases carriage or antibiotic prescription. These results need to be considered when evaluating the overall impact of the use of azithromycin to prevent maternal, neonatal, or infant infections.

REFERENCES


DOI: 10.1093/cid/ciae280


NOTES

Trial status: The trial had been completed at the time of submission of this manuscript.

Author’s contributions: AR, HT, and UDA conceived the trial. AR and PG designed this sub-
study. PG wrote the manuscript and AR contributed significantly to its revision. BC, JDB, IJ, SD, ES, MB, KK, AC HM, and BKJS were involved in the adaptation of the field and laboratory
work. AB, BH, and BS critically reviewed the manuscript. EN was involved with the data
management. CB and TR assisted with the statistical analysis and critically reviewed the
manuscript.

Funding: This work was supported by the Medical Research Council Unit The Gambia at the
London School of Hygiene and Tropical Medicine (MRCG@LSHTM) PhD fellowship. The
main trial was funded by UKRI under the Joint Global Health Trial Scheme (JGHT), reference
number MC_EX_MR/P006949/1 and the Bill and Melinda Gates Foundation reference number
INV-000253.

Acknowledgement: The authors thank all the members of the PregAnZI-2 trial, field,
laboratory and data teams and all staff of the clinical microbiology laboratory of the MRCG @
LSHTM. We also sincerely thank all the participants for agreeing to take part in the study. The
authors acknowledge the following PregAnZI-2 carriage study team members, Fatoumata
Sillah, Nathalie Beloum, Usman N. Nakakana, Madikoi Danso, Joquina C. Jones, Shashu
Graves, Edrissa Sabally, Siaka Badjie, Sulayman Bah, Omar B. Jarra, Abdoulie Suso.

Potential conflicts of interest: All the authors report no conflict of interest.
Figure 1: Study profile

- **Recruited**: n = 500
  - Azithromycin: n = 244
    - Day 0 samples: RVS = 244, NPSm = 242, RS = 240, NPSb = 240
    - Day 6: NPSm = 240, BM = 241, RS = 238, NPSb = 238
    - Day 28*: BM = 222, NPSb = 220, RS = 220
  - Placebo: n = 256
    - Day 0 samples: RVS = 256, NPSm = 255, RS = 252, NPSb = 252
    - Day 6: NPSm = 252, BM = 253, RS = 250, NPSb = 250
    - Day 28*: BM = 243, RS = 240, NPSb = 240
  - Month 4*: BM = 189, NPSb = 188, RS = 188

RVS = rectovaginal swab, NPSm = nasopharyngeal swab (mother), NPSb = nasopharyngeal swab (baby), RS = rectal swab, BM = breast milk.

*aSample collection affected by COVID-19 disruptions.

Figure 2: RS *E. coli* and *K. pneumoniae* carriage and azithromycin resistance

RS = Rectal swabs
AZM = Azithromycin
Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th>Mothers</th>
<th>Azithromycin n = 244</th>
<th>Placebo n = 256</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gambia</td>
<td>122 (50.0)</td>
<td>128 (50.0)</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>122 (50.0)</td>
<td>128 (50.0)</td>
</tr>
<tr>
<td>Age in years(^a), median (interquartile range)</td>
<td>26.0 (21.0-31.0)</td>
<td>26.0 (21.0-30.0)</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandinka</td>
<td>47 (19.3)</td>
<td>50 (19.5)</td>
</tr>
<tr>
<td>Wollof</td>
<td>20 (8.2)</td>
<td>20 (7.8)</td>
</tr>
<tr>
<td>Jola</td>
<td>14 (5.7)</td>
<td>16 (6.3)</td>
</tr>
<tr>
<td>Fula</td>
<td>21 (8.6)</td>
<td>19 (7.4)</td>
</tr>
<tr>
<td>Mossi</td>
<td>115 (47.1)</td>
<td>120 (46.9)</td>
</tr>
<tr>
<td>Gourounsi</td>
<td>5 (2.0)</td>
<td>7 (2.7)</td>
</tr>
<tr>
<td>Peuhl</td>
<td>2 (0.8)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Other</td>
<td>20 (8.2)</td>
<td>23 (9.0)</td>
</tr>
<tr>
<td>Season of delivery(^b), n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry (Nov-May)</td>
<td>137 (56.1)</td>
<td>134 (52.3)</td>
</tr>
<tr>
<td>Wet (Jun-Oct)</td>
<td>106 (43.4)</td>
<td>120 (46.9)</td>
</tr>
<tr>
<td>Mode of delivery, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>238 (97.5)</td>
<td>251 (98.0)</td>
</tr>
<tr>
<td>Caesarean</td>
<td>6 (2.5)</td>
<td>5 (2.0)</td>
</tr>
<tr>
<td>Multiple pregnancy, n (%)</td>
<td>3 (1.2)</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>120 (49.2)</td>
<td>121 (47.3)</td>
</tr>
<tr>
<td>Males</td>
<td>124 (50.8)</td>
<td>135 (52.7)</td>
</tr>
<tr>
<td>Birth Weight(^c) (kg), median (interquartile range)</td>
<td>3.0 (2.8-3.3)</td>
<td>3 (2.8-3.35)</td>
</tr>
</tbody>
</table>

\(^a\)Age missing in n = 52

\(^b\)Season of delivery missing in n = 3

\(^c\)Birth Weight missing in n = 1.
Table 2: Prevalence of *E. coli* carriage and azithromycin resistance in different biological samples from women and their infants

<table>
<thead>
<tr>
<th></th>
<th>Prevalence of carriage</th>
<th></th>
<th>Prevalence of resistance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AZI n/N (%)</td>
<td>Placebo n/N (%)</td>
<td>PR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women - rectovaginal swab samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0(^1)</td>
<td>168/244 (68.9)</td>
<td>173/256 (67.6)</td>
<td>1.02 (0.91-1.15)</td>
<td>0.773</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women - breast milk samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td>9/241 (3.7)</td>
<td>8/253 (3.2)</td>
<td>1.18 (0.46-3.01)</td>
<td>0.808</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td>1/222 (0.5)</td>
<td>2/243 (0.8)</td>
<td>0.55 (0.05-5.99)</td>
<td>1.000</td>
</tr>
<tr>
<td>Month 4</td>
<td>0/189</td>
<td>0/207</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children - rectal swab samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0(^2)</td>
<td>8/240 (3.3)</td>
<td>14/252 (5.56)</td>
<td>0.60 (0.26-1.41)</td>
<td>0.279</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td>150/238 (63.0)</td>
<td>188/250 (75.2)</td>
<td>0.84 (0.75-0.95)</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td>116/220 (52.7)</td>
<td>169/240 (70.4)</td>
<td>0.75 (0.64-0.87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Month 4</td>
<td>140/188 (74.5)</td>
<td>160/206 (77.7)</td>
<td>0.96 (0.86-1.07)</td>
<td>0.479</td>
</tr>
</tbody>
</table>

\(^a\)Antibiotic concentration: azithromycin (0.016-256µg/mL), \(^1\)Samples collected at Day 0, pre-intervention
\(^2\)Samples collected at Day 0, post-intervention
Isolates with MICs ≥32 µg/mL considered resistant based on azithromycin epidemiological cutoff values and limited clinical data for other Enterobacterales
p-values from Fisher's exact test

Table 3: Prevalence of *K. pneumoniae* carriage and azithromycin resistance in different biological samples from women and their infants

<table>
<thead>
<tr>
<th></th>
<th>Prevalence of carriage</th>
<th></th>
<th>Prevalence of resistance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AZI n/N (%)</td>
<td>Placebo n/N (%)</td>
<td>PR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women - rectovaginal swab samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0(^1)</td>
<td>67/244 (27.5)</td>
<td>68/256 (26.6)</td>
<td>1.03 (0.77-1.38)</td>
<td>0.841</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women - nasopharyngeal swab samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0(^1)</td>
<td>4/243 (1.6)</td>
<td>10/254 (3.9)</td>
<td>0.42 (0.13-1.32)</td>
<td>0.175</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td>8/240 (3.3)</td>
<td>2/252 (0.8)</td>
<td>4.2 (0.90-1.07)</td>
<td>0.057</td>
</tr>
</tbody>
</table>

\(^a\)Antibiotic concentration: azithromycin (0.016-256µg/mL), \(^1\)Samples collected at Day 0, pre-intervention
Isolates with MICs ≥32 µg/mL considered resistant based on azithromycin epidemiological cutoff values and limited clinical data for other Enterobacterales
p-values from Fisher's exact test
Women - breast milk samples

<table>
<thead>
<tr>
<th></th>
<th>Women - breast milk samples</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12/241 (5.0)</td>
<td>12/253 (4.7)</td>
<td>1.05 (0.48-1.000)</td>
<td>3/24 (0.4)</td>
<td>1/253 (1.2)</td>
<td>0.472</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td>12/222 (5.4)</td>
<td>7/243 (2.9)</td>
<td>1.88 (0.76-0.241)</td>
<td>1/22 (2)</td>
<td>0/243 (0.5)</td>
<td>0.476</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 4</td>
<td>5/189 (2.6)</td>
<td>3/207 (1.4)</td>
<td>1.83 (0.44-0.487)</td>
<td>0/18 (9)</td>
<td>0/207 (7.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Children - rectal swab samples

<table>
<thead>
<tr>
<th></th>
<th>Children - rectal swab samples</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 02</td>
<td>7/240 (2.9)</td>
<td>8/252 (3.2)</td>
<td>0.92 (0.34-1.000)</td>
<td>0/24 (0.4)</td>
<td>1/252 (0.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td>118/238 (49.6)</td>
<td>93/250 (37.2)</td>
<td>1.33 (1.08-0.006)</td>
<td>14/2 (5.9)</td>
<td>6/250 (2.4)</td>
<td>2.45 (0.96-6.27)</td>
<td>0.067</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td>118/220 (53.6)</td>
<td>79/240 (32.9)</td>
<td>1.63 (1.31-&lt;0.001)</td>
<td>16/2 (20)</td>
<td>5/240 (21)</td>
<td>3.49 (1.30-9.37)</td>
<td>0.012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 4</td>
<td>38/188 (20.2)</td>
<td>47/206 (22.8)</td>
<td>0.89 (0.61-0.543)</td>
<td>4/206 (1.9)</td>
<td>3/18 (8)</td>
<td>0.82 (0.19-3.62)</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Children - nasopharyngeal swab samples

<table>
<thead>
<tr>
<th></th>
<th>Children - nasopharyngeal swab samples</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 02</td>
<td>1/240 (0.4)</td>
<td>0/252 (-)</td>
<td>0.487</td>
<td>0/24 (0)</td>
<td>0/252 (-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td>20/238 (8.4)</td>
<td>16/250 (6.4)</td>
<td>1.32 (0.70-0.393)</td>
<td>0/23 (8)</td>
<td>0/250 (-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td>4/220 (18)</td>
<td>5/240 (2.1)</td>
<td>0.87 (0.24-1.000)</td>
<td>0/22 (0)</td>
<td>0/240 (-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 4</td>
<td>1/188 (0.5)</td>
<td>0/206 (-)</td>
<td>0.477</td>
<td>0/18 (8)</td>
<td>0/206 (-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aAntibiotic concentration: azithromycin (0.016-256µg/mL).  
*bSamples collected at Day 0, pre-intervention  
**Samples collected at Day 0, post-intervention  
Isolates with MICs ≥32 µg/mL considered resistant based on azithromycin epidemiological cutoff values and limited clinical data for other Enterobacterales  
*p-values from Fisher’s exact test