# Antibiotic susceptibility profile of bacterial pathogens isolated from febrile children under 5 years of age in Nanoro, Burkina Faso

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## Abstract

**Background:** The curative power of antimicrobials is severely threatened due to emerging resistance to first-line antibiotics worldwide. With a limited reserve of antibiotics, increasing antimicrobial resistance has become a global concern, but there is a paucity of such data in Burkina Faso, and the West African region in general. Therefore, this study aims to determine the antibiotic susceptibility profile of bacterial species isolated from febrile children under 5 years of age in Nanoro (Burkina Faso).

**Methods:** Clinical specimens (blood, stool, and urine) were collected from 1099 febrile children attending the peripheral health facilities and the referral hospital in Nanoro. Bacterial isolates from these clinical specimens were assessed for their susceptibility against commonly used antibiotics by standard disc diffusion procedure and minimal inhibitory concentration method (when appropriate).

**Results:** In total, 141 bacterial strains were recovered from 127 febrile children of which 65 strains were isolated from blood, 65 from the stool, and 11 from urine. Predominant bacterial isolates were *Salmonella species* (56.7%; 80/141) followed by *Escherichia coli* (33.3%; 47/141). Antibiotic susceptibility testing revealed *Salmonella* species were highly resistant to ampicillin (70%; 56/80), trimethoprim-sulfamethoxazole (65%; 52/80), and chloramphenicol (63.8%; 51/80). *E. coli* isolates were highly resistant to trimethoprim-sulfamethoxazole (100%), ampicillin (100%), ciprofloxacin (71.4%; 10/14), amoxicillin-clavulanate (64.3%; 9/14), ceftriaxone (64.3%; 9/14), and gentamycin (50%; 7/14). Moreover, 7 out of 14 *E. coli* isolates were producers of the ß-lactamase enzyme, suggesting multi-drug resistance against b-lactam as well as non-b-lactam antibiotics. *S. pneumoniae* isolates were fully resistant to tetracycline and 50% to penicillin G. Multi-drug resistance was observed in 54.6% (59/108) of the isolates of which 56 (54.9%) were Gram-negative bacteria and 3 (50.0%) Gram-positive bacteria.

**Conclusions:** The antibiotic susceptibility profiling showed an alarming high resistance to commonly used antibiotics to treat bacterial infections in the study region. The work prompts the need to expand antibiotic resistance surveillance studies in Burkina Faso, and probably the whole region (West Africa).


## Background

The development of antibiotics against bacterial infections has been one of the greatest achievements of modern medicine (1–5). However, the efficacy of these antibiotics is not endless and this success is now being jeopardized by the increasing occurrence of antibiotic resistance (ABR). Nowadays, ABR is considered as one of the most important threats to public health and one of the biggest health challenges that mankind faces (6–11). ABR is strongly associated with increased risk of infection severity, patient morbidity and mortality rate, prolonged hospitalization time and healthcare costs (12). One of the main obstacles in low-and middle-income countries is the lack of practical tools in primary healthcare facilities to reliably differentiate bacterial infections from other febrile infections. As a result, antibiotics are systematically prescribed without any evidence-base, thereby significantly contributing to increasing ABR (13).

To solve this alarming situation, the World Health Organization (WHO) has developed a global antimicrobial resistance (AMR) action plan that encompasses reinforcing AMR knowledge through surveillance and research (12). A better understanding of local AMR patterns is crucial to firstly guide clinical management of infectious diseases and secondly for the early detection of ABR to first-line antibiotics used in primary healthcare facilities. However, the information about the true extent of the antibiotic resistance threat in the African region is limited to 6 out of 47 countries where studies on AMR have been performed. The resulting gap in monitoring AMR weakens decision-making on antibiotic resistance policy (14, 15).

The same applies to Burkina Faso, where studies have revealed a worrying resistance against several commonly prescribed first-line antibiotics in primary healthcare facilities such as amoxicillin, amoxicillin-clavulanic acid and ampicillin (9, 10, 16–18). These studies highlight that significant resistance is recorded for several bacterial species, which have spread into hospitals and communities. It has for example been observed that nurses providing the first-line care in primary healthcare facilities use the 10 years old national treatment recommendations (18), but this guideline does not contain up to date information about the resistance profiles of different circulating bacterial strains and species. In addition, this guideline is mostly based on findings in high income countries and does not necessarily reflects the best treatment options for low income countries such as Burkina Faso. Furthermore, this situation is exacerbated due to the fact that the general public has (without a proper prescription) access at local markets and shops to antibiotics, where supply and quality of drugs are not appropriately controlled. This does not only threat the effectiveness of current first-line antibiotic treatments used in peripheral health facilities, but also second and third-line antibiotics (6, 19). There are currently no structural mechanisms in place in Burkina Faso to monitor antibiotic use and the susceptibility of bacteria to available antibiotics. The existing sentinel sites for antibiotic resistance surveillance are mainly in tertiary urban hospitals and often not operational.

The present study aims to fill part of the gap in our knowledge on the current effectiveness of antimicrobials by presenting the antibiotic susceptibility profile of bacteria isolated from various clinical specimens of febrile children less than 5 years of age in the Nanoro health district, Burkina Faso. Among the antibiotics tested in this study are several that are recommended as the first-line antibiotics by the Ministry of Health (MoH) of Burkina Faso to treat various bacterial infections (Table 1). According to this guideline sepsis/suspected bacterial bloodstream infections (bBSIs) and suspected pneumonia are treated with ampicillin (AMP) and gentamycin (GEN). In the cases of suspicion of typhoid fever, it is recommended to treat the infection with the ciprofloxacin (CIP). Furthermore, it is advised to treat suspected simple pneumonia with the trimethoprim-sulfamethoxazole (SXT) (18). For suspected cases of bacterial gastroenteritis (bGE), the first-line antibiotic is also CIP and for suspected bacterial urinary tract infections (bUTIs) either SXT or amoxicillin (AMOX) are used (18). The first-line therapy for the treatment of the meningitis infections is chloramphenicol (CL) and AMP; in case CL appears to be ineffective, ceftriaxone (CRO) is used as second line-treatment (18).

Table 1
Antibiotic categories and antibiotic agents used for susceptibility testing

| Antibiotic categories | Antibiotic agents | Disc content | E-test content |
|---|---|---|---|
| Extended-spectrum cephalosporin; 3rd generation cephalosporin | Ceftriaxone (CRO)* | 30 µg | 0.016-256 mg/L |
| | Ceftazidime (CAZ) | 30 µg | - |
| Cephamycins | Cefoxitin (FOX) | 30 µg | - |
| Penicillin* | Ampicillin (AMP)* | 10 µg | 0.016-256 µg/L |
| | Penicillin(PEN) | 10 µg | - |
| Penicillin+ß-lactamase inhibitor | Amoxicillin-clavulanate (AMC)* | 20/10 µg | - |
| Trimethoprim and sulfamide combination (Folate pathway inhibitors) | Trimethoprim-sulfamethoxazole (SXT)* | 1.25/23.75 µg | - |
| | | | - |
| Aminoglycosides | Gentamycin (GEN)* | 10 µg | - |
| | Amikacin (AK) | 30 µg | - |
| Quinolone and fluoroquinolones | Ciprofloxacin (CIP)* | 5 µg | - |
| | Nalidixic acid (NA) | 30 µg | - |
| | Norfloxacin (NOR) | 30 µg | - |
| Carbapenems | Ertapenem (ETP) | 10 µg | - |
| | Imipenem (IPM) | 10 µg | 0.02-32 mg/L |
| Macrolides | Azithromycin (AZI) | 15 µg | - |
| | Erythromycin (ERY)* | 15 µg | - |
| Phenicols | Chloramphenicol (CL)* | 30 µg | - |
| Lincosamides | Clindamycin (CC) | 2 µg | - |
| Glycopeptides | Vancomycin (VAN) | 30 µg | 0.016-256 µg/L |
| Tetracyclines | Tetracycline (TET) | 30 µg | - |
| Nitrofurans | Nitrofurantoin (NI) | 30 µg | - |

**= first- line treatment proposed by the Ministry of Health of Burkina Faso to treat bacterial infections.* This guideline recommends to treat sepsis (or suspected bacterial bloodstream infections) suspected pneumonia with Ampicillin (AMP) or Gentamycin (GEN). In the case of suspicion of typhoid fever Ciprofloxacin (CIP) is indicated and Trimethoprim-sulfamethoxazole (SXT) is used to treat simple pneumonia (18). For suspected cases of bacterial gastroenteritis CIP is used and for suspected bacterial urinary tract infection either SXT or amoxicillin (AMOX) is used (18). Chloramphenicol (CL) and AMP are mostly used as first-line therapy for bacterial meningitis and Ceftriaxone (CRO) as second line-treatment (18).

# Methods

### Patients and clinical samples

The present study was conducted in the framework of a larger project that was investigating the aetiologies, diagnoses, and treatment of febrile children in the Health district of Nanoro. For the present study, any child under-5 years of age attending one of the four primary healthcare facilities or the referral hospital of the Health district of Nanoro with documented fever (axillary temperature $\geq$ 37.5°C) was invited to participate in the study. Cases were managed by health facility or referral hospital staff according to the Burkinabe national protocol of diseases management based on the Integrated Management of Childhood Illness (IMCI) (20). Furthermore, clinical specimens (blood, stool and urine) were collected at enrolment for microbiological analyses and antibiotic susceptibility testing, at the laboratory of Microbiology of the Clinical Research Unit of Nanoro (CRUN). In case the children could not produce a urine or stool sample at the time of enrolment, sterile containers were provided to the legal guardian to collect these samples at home and return them as soon as possible to the health facility within 48 hours after inclusion.

Written informed consent was obtained from parents or legal guardians before any data and specimen collection. The study protocol was reviewed and approved by the National Ethical Committee for Health Research, Burkina Faso (Deliberation No. 2014-11-130).

### Laboratory procedures

#### *Sample collection and bacterial species identification*

The microbiology procedures used in this study have been already described in detail elsewhere (21). Briefly, from each child, 1-3 mL of venous blood was collected into a paediatric blood culture bottle (BD BACTEC Peds Plus$^{TM}$/F culture vials, Becton Dickinson and Company, Sparks, Maryland, USA) at enrolment. These bottles were incubated at 35°C ± 2°C in an automated incubator BACTEC 9050 (Becton Dickinson and Company, Sparks, Maryland, USA) for a maximum of 5 days as recommended by the manufacturer. Positive bottles were Gram stained and further sub-cultured on 5% fresh sheep blood agar (SBA),

chocolate agar with PolyViteX (PVX) or IsoVitaleX (IVX), and Gram-negative selective agar (Eosin Methylene Blue (EMB) agar or Mac Conkey agar) and incubated at 35°C ± 2°C for 18-24 hours. The pathogens present in positive blood cultures were identified by standard microbiological methods (22-24). In addition, the Analytical Profile Index (API; bioMerieux Marcy-L'Etoile, France) 20E system was used for biochemical identification. Identified *Salmonella* strains were further serotyped using Remel™ agglutinating sera (Thermo Scientific™, United Kingdom) to detect *Salmonella* O and H antigens (25).

Fresh stool samples collected in sterile containers were inoculated in *Salmonella* enrichment broth (Sodium Selenite broth), on Hektoen and EMB (only used for children under 2 years) agars and incubated at 35°C ± 2°C for 18-24 hours. After 4-6 hours, the sodium selenite broth was sub-cultured on *Salmonella-Shigella* (*SS*) agar and incubated at 35°C ± 2°C for 18-24 hours. Suspect colonies sought for were *Salmonella* species, *Shigella* species, and enteropathogenic *Escherichia coli* (EPEC), only for children under 2 years). Suspect colonies were further identified according to standard microbiological methods. Identified suspected strains were also serotyped by slide agglutination (Bio-Rad antisera, Marnes-la-Coquette, France).

Midstream urine samples were collected in sterile containers and screened with a urine dipstick test (Urocolor, Standard Diagnostics Inc, Suwan, South Korea). If leucocytes and nitrite were present (indicating a probable urinary infection), the urine samples were plated on appropriate agar (cysteine-lactose-electrolyte-deficient [CLED] and EMB agars) and incubated for 18-24 hours at 35°C ± 2°C. A pure bacterial growth of $^{3}10^{5}$ colonies forming units (CFU)/mL was considered as significant bacteriuria according to the Stamm and Kass recommendation (26).

### *Antimicrobial susceptibility testing*

Antimicrobial susceptibility testing of bacterial isolates was done using the standard disc diffusion procedure (Kirby-Bauer) and the Minimal Inhibitory Concentration (MIC) as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (27, 28). Antibiotic susceptibility was determined for *non-typhoidalSalmonella (NTS), typhoidal Salmonella (TS), E. coli, Shigella flexeneri, Shigella dysenteriae, Enterobacter agglomerans, Haemophilus Influenza* b*, Neisseria meningitidis, Streptococcus pneumoniae,* and *Staphylococcus aureus.* Antibiotic susceptibility testing of isolated EPEC was not done, because according to general practice cares as well as local standard practice like applied in Burkina Faso, gastroenteritis caused by these bacteria is not treated with antibiotics (18, 29).

A suspension of each bacterial isolate to be tested was prepared to the turbidity of 0.5 McFarland standard (measured by BD PhoenixSpec, Nephelometer Becton Dickinson and Company, Sparks, Maryland, USA). The 0.5 McFarland standard bacterial suspension was subsequently plated out on appropriate agar (plate of 100 mm diameter) depending on the bacterial species studied. A maximum of 6 antibiotic discs and a maximum of 3 plastic E-test strips were deposited per plate. The inoculate agars with antibiotic discs or E-tests were incubated for 16-18 hours at 35°C ± 2°C according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (27, 28). The diameter of the inhibition zone was measured and recorded in millimetres for each disc and in microgram per millilitre (mg/mL) for each E-test. The results of antibiotic susceptibility testing were interpreted according to the criteria of the CLSI (27, 28). The antibiotic discs (BD Seni-Disc™, Becton Dickinson and Company, B.V., Vianen, The Netherlands) used for antibiotic susceptibility testing as well as the minimal inhibition concentration (MIC; E-tests; Liofilchem S.r.l, Roseto degli Abruzzi(TE), Italy) are reported in table 1.

### *Determination of Extended Spectrum beta-lactamase producers*

The extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* were determined by using both ceftazidime (CAZ) (30µg) and cefotaxime (CTX) (30µg) discs, alone or in combination with clavulanate (C) (10 µg) discs, as described in CLSI (27, 28). A bacteria strain was considered as potential ESBL-producer, when the inhibition zone diameters were ≤25mm for ceftriaxone (CRO) disc, ≤22mm for CAZ disc, and ≤27mm for CTX disc (27, 28). An *Enterobacteriaceae* phenotype is indisputably considered to be an ESBL producing phenotype bacterium if the difference between the inhibition zone diameter for either antibiotic tested in combination (CAZ + C) or (CTX + C) and the inhibition zone diameter of the corresponding antibiotic tested alone (CAZ or CTX) is ≥ 5 mm (27, 28). *S. aureus* species were considered as methicillin-resistant *Staphylococcus aureus* (MRSA) strains when the inhibition zone diameter of cefoxitin disc (FOX; 30 $\mu$g) on Mueller Hinton (MH) agar plate is ≤21 mm after 16–18 hours of incubation, according to CLSI guidelines (27, 28).

An isolate was considered to be multi-resistant when it is resistant to at least one antibiotic agent in each of all three antibiotic categories used for therapy or prophylaxis based on Burkina Faso national treatment guidelines.

### *Quality control*

Standard bacteriological procedures were performed in accordance with standard operating procedures (SOPs) of the CRUN microbiology department, to ensure the conformity of microbiology laboratory tests. Monthly internal quality controls are performed and the CRUN laboratory is subjected to external quality control organised by the World Health Organization (WHO) and National Institute for Communicable Diseases (NICD) according to a standard auditing protocol. American Type Culture Collection (ATCC ®) standard reference strains *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC® 14028™, *Staphylococcus aureus* ATCC® 25923™, *Staphylococcus epidermidis* ATCC® 14990™, *Streptococcus pyogenes* ATCC® 19615™, *Enterococcus faecalis* ATCC® 29212™, and *Streptococcus pneumonia* ATCC® 49619™ were used for internal quality control.

### *Data analysis*

The inhibition diameters for each antibiotic tested for each investigated bacterium were recorded using Excel 2016. These data were double entered by 2 independent technicians and subsequently validated by the lab-manager. Data analysis was performed using STATA version 13 software. For the interpretation of the resistant rate of strains identified in the present study, the following classification was used: low (resistance rate <20%), moderate (resistance from 20 to 50%), high (resistance rate from 50 to 75%), alarming (resistance rate from 75 to 100%) for the antibiotics tested (30, 31).

## Results

# Study population characteristics

The characteristics of the study population are presented in Table 2. In total 1099 children participated in the study of whom 55.2% were male and 44.8% female. One hundred and twenty-seven (11.6%) of the febrile children had one (or more) confirmed bacterial infection(s). In total, 1099 blood samples (100%), 757 (68.9%) stool samples and 739 (67.2%) urine samples were collected for microbiology analyses. Among them, a total of 141 bacterial strains were identified. Out of these bacterial strains, 65 were confirmed in bacterial bloodstream infections (bBSI), 65 in bacterial gastroenteritis (bGE), and 11 in bacterial urinary tract infections (bUTI) (Table 2).

Table 2
Basic characteristics of the study population.

| Characteristic | Study population | Confirmed Bacterial infection | Laboratory confirmed bacterial Infections | | | Bacterial co-infections | | | |
|---|---|---|---|---|---|---|---|---|---|
| | N = 1099 | Yes | bBSI | bGE | bUTI | bBSI associated to bGE | bBSI associated to bUTI | bGE associated to bUTI | bBSI associated to bGE and bUTI |
| Demographic data | | | | | | | | | |
| Total, n (%) | 1099 (100.0) | 127 (11.6) | 65 (5.9) | 65 (5.9) | 11 (1.0) | 11 (1.0) | 2 (0.2) | 3 (0.3) | 2 (0.2) |
| Male, n (%) | 607 (55.2) | 59 (9.7) | 34 (52.3) | 28 (43.1) | 5 (45.5) | 6 (54.5) | 1 (50.0) | 2 (66.7) | 1 (50) |
| Female, n (%) | 492 (44.8) | 68 (13.8) | 31 (47.7) | 37 (56.9) | 6 (54.5) | 5 (45.5) | 1 (50.0) | 1 (33.3) | 1 (50) |
| Age ≤ 12 months (%) | 306 (27.8) | 33 (10.8) | 16 (24.6) | 16 (24.6) | 5 (45.5) | 5 (45.5) | 0 (0) | 1 (33.3) | 0 (0) |
| Age > 12 months (%) | 793 (72.20) | 94 (11.8) | 49 (75.4) | 49 (75.4) | 6 (54.5) | 6 (54.5) | 2 (100) | 2 (66.7) | 2 (100) |

*bBSI: bacterial bloodstream infection; bGE: bacterial gastroenteritis; bUTI: bacterial urinary tract infection.*

The distribution of the bacterial isolates according to the types of infection is summarized in Table 3. In total 135 Gram-negative bacteria strains were isolated amongst which *Salmonella species* (59.3%; 80/135) were the most prevalent. Only a few Gram-positive bacteria were isolated from blood, with *Streptococcus pneumoniae* (66.7%; 4/6) as the most frequent species.

Table 3
Distribution of the identified bacterial isolates according to the site of infection.

| Isolated bacteria | bBSI | bGE | bUTI | bBSI + bGE | | bBSI + bUTI | | bGE + bUTI | |
|---|---|---|---|---|---|---|---|---|---|
| | n (%) | n (%) | n (%) | Blood n (%) | Stool n (%) | Blood n (%) | Urine n (%) | Stool n (%) | Urine n (%) |
| Gram-negative bacteria | | | | | | | | | |
| *NTS (76)* | 47 (72.3) | 29 (44.6) | - | 10 (90.9) | 10 (90.9) | 1 (50) | - | 2 (66.7) | - |
| *TS (4)* | 4(6.1) | - | - | - | - | - | - | - | - |
| *E. coli (47)* | 4 (6.1) | 33 (50.8) | 10 (90.9) | 1 (9.9) | 1 (9.9) | 1 (50) | 2 (100) | 1 (33.3) | 3 (100) |
| *Klebsiella species (1)* | - | - | 1 (9.1) | - | | - | - | - | - |
| *N. meningitidis (2)* | 2 (3.1) | - | - | - | | - | - | - | - |
| *Shigella species (3)* | - | 3 (4.6) | - | - | | - | - | - | - |
| *H. influenzae* b (1) | 1 (1.5) | - | - | - | | - | - | - | - |
| *E. agglomerans (1)* | 1 (1.5) | - | - | - | | - | - | - | - |
| Gram-positive cocci | | | | | | | | | |
| *S. aureus* (2) | 2 (3.1) | - | - | - | | - | - | - | - |
| *S. pneumoniae* (4) | 4 (6.1) | - | - | - | | - | - | - | - |
| Total (141) | 65 (46.1) | 65 (46.1) | 11 (7.8) | 11 (16.9) | 11 (16.9) | 2 (0.8) | 2 (18.2) | 3 (4.6) | 3 (27.3) |

*The site of infections i.e. blood, gastro-intestinal tract, and urinary tract; ; NTS: non-typhoidal Salmonella; TS. typhoidal Salmonella; bBSI: bacterial bloodstream infection; bGE: bacterial gastroenteritis; bUTI: bacterial urinary tract infection; - : not found, bBSI + bGE: bacterial bloodstream infection associated with bacterial gastroenteritis; bBSI + bUTI: bacterial bloodstream infection associated with bacterial urinary tract infection; bGE + bUTI: bacterial gastroenteritis associated with bacterial urinary tract infection.*

# Antibiotic susceptibility testing

# Antibiotic susceptibility of Gram-negative bacteria

The antibiotic susceptibility testing was performed on 102 Gram-negative bacteria, but not on 33 EPEC isolates from stool. The antibiotic susceptibility results of 102 Gram-negative bacteria isolates recovered from the various clinical specimen are presented in Table 4. The analysis of the susceptibility patterns of predominant Gram-negative bacteria isolates such as non-typhoid *Salmonella* (*NTS*) and *E. coli* revealed a resistance rate varying between high to alarming for several antibiotics tested (Table 4). For example, among *NTS* isolated from blood and stools, the rate of reported resistance was alarming or moderated to CL, respectively. However, for *NTS*, the susceptibility testing revealed a low resistance rate for ciprofloxacin (CIP) and nalidixic acid (NA); (Table 4). In contrast, for *E. coli* isolated from urine, an alarming resistance rate was reported to CIP and NA. In addition, 7 strains of *E. coli* produced β-lactamase of which 6 were isolated from urine, suggesting multi-drug resistance against β-lactam and non-β-lactam antibiotics. For other Gram-negative bacteria, two out of four strains of *typhoidal Salmonella* (TS) showed high resistance to trimethoprim-sulfamethoxazole (SXT; 50%) and nalidixic acid (NA; 50%). All *N. meningitidis* strains tested had an alarming resistance to SXT and one was resistant to penicillin (PEN). Less frequently isolated *E. agglomerans* (0.7%) and *H. influenzae* b (0.7%) were found to be sensitive to most of the antibiotics tested, except for trimethoprim-sulfamethoxazole (SXT; 100% resistance) to *H. influenzae* b. The single *Klebsiella* species isolated from urine was fully resistant to SXT (100%).

Table 4
Antibiotic susceptibility profiling of different bacteria strains isolated from various clinical specimens.

| | Infection site | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Blood | | | | | | | | Stool | | Urine | |
| Bacteria species, (N) | *NTS (47)* | *TS (4)* | *E. coli (4)* | *N. m (2)* | *E. agglomerans (1)* | *S. p (4)* | *S. aureus (2)* | *Hi b (1)* | *NTS (29)* | *Shigella species (3)* | *E. coli (10)* | *Klebsiella species (1)* |
| *Antibiotics*, n (%) | | | | | | | | | | | | |
| SXT* | 37 (78.7) | *2 (50)* | 4 (100) | 2 (100) | 0 (0) | *2 (50)* | 0 (0) | *1 (100)* | 13 (44.8) * | 1 (33.3) * | 10 (100) * | 1 (100) * |
| AMP* | 43 (91.5) * | 0 (0) | 4 (100) * | 0 (0) | 0 (0) | 0 (0) | - | 0 (0) | 13 (44.8) | 3 (100) | 10 (100) * | 1 (100) * |
| AMC | 10 (21.3) * | 1 (25) * | 2 (50) * | - | 0 (0) | - | - | - | 7 (24.1) | 1 (33.3) | 7 (70) * | 0 (0) * |
| CRO* | 0 (0) | 0 (0) | 2 (50) * | 0 (0) * | 0 (0) | 0 (0) * | - | 0 (0) * | 0 (0) | 0 (0) | 7 (70) | 0 (0) |
| CL | 38 (80.8) | 2 (50) | 0 (0) | 0 (0) | 0 (0) | 1 (25) | 0 (0) | - | 11 (37.9) | 1 (33.3) | 0 (0) | 0 (0) |
| CIP* | 0 (0) | 0 (0) | 2 (50) | 0 (0) | 0 (0) | - | 0 (0) | 0 (0) | 2 (6.9) * | 0 (0) * | 8 (80) | 0 (0) |
| NA | 4 (8.5) | 2 (50) | 2 (50) | - | 0 (0) | - | - | - | 2 (6.9) | 0 (0) | 8 (80) | 0 (0) |
| GEN* | - | - | 2 (50) * | - | 0 (0) | - | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 5 (50) | 0 (0) |
| AK | 0 (0) | 0 (0) | 0 (0) | - | 0 (0) | - | 0 (0) | - | 0 (0) | 0 (0) | 1 (10) | 0 (0) |
| CAZ | 0 (0) | 0 (0) | 2 (50) | - | 0 (0) | - | - | - | 0 (0) | 0 (0) | 6 (60) | 0 (0) |
| IPM | 0 (0) | 0 (0) | 0 (0) | - | 0 (0) | 0 (0) | - | - | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| ETP | 0 (0) | 0 (0) | 0 (0) | - | 0 (0) | - | - | - | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| PEN | - | - | - | 1 (50) | - | 2 (50) | 2 (100) | - | - | | - | |
| ERY | - | - | - | - | - | 0 (0) | 1 (50) | - | - | | - | |
| TET | - | - | - | - | - | 4 (100) | 1 (50) | - | - | | - | |
| CC | - | - | - | - | - | 0 (0) | 1 (50) | - | - | | - | |
| NOR | - | - | - | - | - | - | 0 (0) | - | - | | - | |
| NI | - | - | - | - | - | - | 0 (0) | - | - | | - | |
| VAN | - | - | - | - | - | 0 (0) | 0 (0) | - | - | | - | |
| AZI | - | - | - | - | - | 0 (0) | - | - | - | | - | |

*N (%)*: *the prevalence of resistance phenotypes is presented in percentage*; *NTS*: *non-typhoid Salmonella; TS*: *typhoidal Salmonella ; Nm*: *Neisseria meningitidis ; Sp : Streptococcus pneumoniae ; Hib : haemophilus influenzae* b ; *CRO* : *ceftriaxone; AMC*: *amoxicillin-clavulanate; AMP*: *ampicillin; GEN*: *gentamycin; SXT*: *trimethoprim-sulfamethoxazole; CIP*: *ciprofloxacin; NA*: *nalidixic acid; CL*: *chloramphenicol; ERY*: *erythromycin; CC*: *clindamycin; TET*: *tetracycline; PEN*: *penicillin; OX*: *oxacillin; IMP* : *imipenem ; ETP* :*ertapenem ; NOR* : *norfloxacin ; NI* : *nitrofurantoin ; AZI* : *azithromycine ; CAZ* : *ceftazidim; AK* : *amikacin .- : not tested; *: first- line treatment proposed by the Ministry of Health of Burkina Faso to treat these infections*.

In Table 5 the resistance rates to the first-line therapies as recommended by MoH of Burkina Faso are presented in more detail. The resistance rates of bacteria associated with bacterial gastroenteritis were in the general low to moderate. However, in the case of bacterial urinary tract infections, *E. coli* and *Klebsiella* resistance rate against SXT was 100%. AMP is commonly used to treat invasive bacterial infections, but high to alarming resistance rates were found in the present study. In contrast, CRO seemed to remain effective against *NTS*.

Table 5
Resistance rates of bacteria species isolated to first-line antibiotics used in Burkina Faso*.

| | Infection type | | | | | | |
|---|---|---|---|---|---|---|---|
| | bBSI | | | bGE | | bUTI | |
| Antibiotic, n (%) | AMP | GEN | CRO | SXT | CIP | SXT | AMP |
| Isolated bacteria (N) | | | | | | | |
| *NTS* (76) | 43 (56.6) | 1 (1.3) | 0 (0) | 13 (17.1) | 2 (6.9) | - | - |
| *E. coli* (14) | 4 (100) | 2 (50) | 2 (50) | - | - | 10(100) | 10 (100) |
| *N.m* (2) | 0 (0) | 0 (0) | 0 (0) | - | - | - | - |
| *Shigella sp.* (3) | - | - | - | 1 (33.3) | 0 (0) | - | - |
| *Klebsiella sp.* (1) | - | - | - | - | - | 1 (100) | 1 (100) |
| *S. p* (4) | 0 (0) | 0 (0) | 0 (0) | - | - | - | - |
| *S. aureus* (2) | - | 0 (0) | 0 (0) | - | - | - | - |

*bBSI: bacterial bloodstream infections (blood stream infections and meningitis); bGE: bacterial gastroenteritis; bUTI: bacterial urinary infection; NTS: non-typhoid Salmonella; Nm: Neisseria meningitidis; CRO: ceftriaxone; AMP: ampicillin; GEN: gentamycin; SXT: trimethoprim-sulfamethoxazole; CIP: ciprofloxacin; AMOX: amoxicillin S. p: Streptococcus pneumoniae; -: not found; *: first- line treatment proposed by the Ministry of Health of Burkina Faso to treat these infections.*

Importantly, CRO showed to be also effective against *N. meningitides* and *H. influenzae* b that are often incriminated in meningitis epidemics in Burkina Faso, which is located in Lapeyssonnie's belt.

## Antibiotic susceptibility of Gram-positive cocci

The antibiotic susceptibility results of the 6 Gram-positive cocci strains isolated are presented in Tables 4 and 5. Out of four *Streptococcus pneumoniae*, two isolates were resistant to 2 of the first-line antibiotics tested (PEN and SXT). The 2 *Staphylococcus aureus* recovered were both resistant to PEN and one against ERY.

In addition to first-line antibiotic tested, all *S. pneumoniae* isolates showed resistance to TET (100%). As for *S. aureus*, 1 out of 2 was resistant to clindamycin (CC) and tetracycline (TET). In contrast, CRO that is used as the first-line antibiotic to treat bacterial meningitis showed to be effective against *S. pneumoniae*.

## Resistance profiling of invasive bacteria isolated from multiple infections

The resistance profiling results of bacterial isolates obtained from different clinical specimen from the same study case are presented in Table 6. In total, 11 bacterial isolates (10 *NTS* and 1 *E. coli*) were identified simultaneously in blood and stool. The resistance rate of *NTS* strains identified from both infection sites was alarming to the first-line antibiotics (AMP and SXT) tested. Importantly, 2 children had three types of different infections; one child had an *E. coli* strain responsible for bBSI, bGE, and bUTI, and in another child, 2 *NTS* strains were responsible for bBSI and bGE, and one *E. coli* caused bUTI. Overall, all these bacteria identified from these 3 sites of infections were fully resistant to AMP and SXT, which are commonly used as first-line antibiotics to treat these infections cases.

Table 6
Resistance rate to recommended first-line therapy* for the treatment of bacterial multiple infections identified

| | | bBSI + bGE (11) | | | | | bBSI + bUTI (2) | | | | bGE + UTI (3) | | bBSI + bGE + bUTI (2) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolated bacteria | Infection site | AMP | GEN | CRO | SXT | CIP | AMP | GEN | CIP | SXT | SXT | CIP | AMP | GEN | CRO | SXT | C |
| | | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n |
| *NTS* | Blood (10) | 9(90) | 0 (0) | 0 (0) | 8 (80) | 5 (50)[a] | 1 (100) | 0 (0) | 0 (0) | 1 (100) | NA | NA | 1 (100) | 0 (0) | 0 (0) | 1 (100) | 0 |
| | Stool (10) | 9 (90) | 0 (0) | 0 (0) | 9 (90) | 2 (20)[b] | NA | NA | NA | NA | 1 (50) | 0 (0) | 1 (100) | 0 (0) | 0 (0) | 1 (100) | 0 |
| *E. coli* | Blood (1) | 1 (100) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 1 (100) | 0 (0) | 0 (0) | 1 (100) | NA | NA | 1 (100) | 0 (0) | 0 (0) | 1 (100) | 0 |
| | Stool (1) | nAST | nAST | nAST | nAST | nAST | NA | NA | NA | NA | nAST | nAST | nAST | nAST | nAST | nAST | n |
| | Urine (3) | NA | NA | NA | NA | NA | 2 (100) | 0 (0) | 0 (0) | 2 (100) | 3 (100) | 1 (33.3) | 2 (100) | 0 (0) | 0 (0) | 2 (100) | 0 |

*bBSI + bGE: bacterial bloodstream infection associated with bacterial gastroenteritis; bBSI + bUTI: bacterial bloodstream infection associated with bacterial urinary tract infection; bGE + bUTI: bacterial gastroenteritis associated with bacterial urinary tract infection; bBSI + bGE + bUTI: bacterial bloodstream infection associated with bacterial gastroenteritis; and bacterial urinary tract infection; NTS: non-typhoid Salmonella; CRO: ceftriaxone; AMP: ampicillin; GEN: gentamycin; SXT: trimethoprim, -sulfamethoxazole; CIP: ciprofloxacin; NA: not applicable; nAST = no antibiotic susceptibility testing; *= first- line treatment proposed by the Ministry of Health of Burkina Faso to treat these infections. a, b = the antibiotic susceptibility results of NTS strains are interpreted as Intermediate.*

# Multi-drug resistant (MDR) bacteria

In total, 56/102 (54.9%) of Gram-negative and 3/6 (50%) Gram-positive bacteria were MDR (Table 7). Ten out of fourteen (71.4%) *E. coli* strains isolated in this study revealed resistance to SXT, AMP and CIP. For *Salmonella* species, 56.3% (45/80) of the strains isolated were resistant to SXT, AMP and CL. These antibiotics are recommended by national treatment guidelines of Burkina Faso to treat the infections found in this study (Table 5).

Table 7
Frequency of multi-drug resistant (MDR) bacterial isolates from various clinical specimens.

| Isolated bacteria | Total number of isolates | MDR n (%) |
|---|---|---|
| Gram-negative bacteria | **102** | **56 (54.9)** |
| *NTS* | 76 | 44 (57.9) |
| *TS* | 4 | 1 (25) |
| *E coli*[*] | 14 | 10 (71.4) |
| *N meningitidis Y/W135* | 2 | 0 |
| *Shigella species* | 3 | 1 (33.3) |
| *H influenza b* | 1 | 0 |
| *E agglomerans* | 1 | 0 |
| *Klebsiella species* | 1 | 0 |
| Gram-positive cocci | **6** | **3 (50.0)** |
| *S aureus* | 2 | 1(50.0) |
| *S pneumoniae* | 4 | 2 (50.0) |
| Total | **108** | **59 (54.6)** |

*These bacteria were isolated from blood, stool, and urine samples collected in children under 5;* MDR: *Multi-drug resistant;* NTS: *non-typhoidal Salmonella;* TS: *typhoidal Salmonella; *: Sub-population of E. coli strains isolated from blood and urine*

## Discussion

The study revealed worryingly resistance rates to first-line antibiotics commonly prescribed in Burkina Faso to treat bloodstream infections, bacterial gastroenteritis, and bacterial urinary infections. For example, according to the treatment guidelines of the MoH of Burkina Faso (18), sepsis/suspected bacterial bloodstream infections (bBSIs) caused by *E. coli* or *NTS* are treated with ampicillin (AMP), but resistance rates were found to be almost 100% in the present study. This observation is in line with other studies from the same study area (17) and other sub-Sahara African countries who also reported alarming resistance of *E. coli* and *NTS* to first-line antibiotics (32–35). Urinary tract infections suspected to be caused by *E. coli* or *Klebsiella* species are treated with trimethoprim-sulfamethoxazole (SXT) or amoxicillin (AMOX) (same antibiotic category as AMP), but these first-line antibiotics for UTI treatment revealed a resistance rate of 100% in this study. Although low resistance of *NTS* to CIP (fluoroquinolones) was found, the efficacy of this antibiotic must be carefully monitored as it is widely used to treat bacillary dysenteries by children under 5 years in West Africa (18, 36).

The present work also reported that 85.7% of *E. coli* isolates from the urine of these children were β-lactamase enzyme producers. This is worrying as these strains (producing β-lactamase) usually show co-resistance to non-β-lactam antibiotics, such as aminoglycosides and fluoroquinolones (37–39). This explains the high resistance of *E. coli* isolated from urine to β-lactam and cross-resistance to non-β-lactam antibiotics reported in this study. It was reported that this enzyme is predominately produced by bacteria that are multi-resistant to the group of β-lactam antibiotics (1, 37, 39, 40), which are frequently used to treat infections caused by Gram-negative bacteria like *Enterobacteriaceae*. This observation, supported by data form another study from Burkina Faso (10) and from other African countries (15, 33, 41, 42), implies that treatment options for bacterial diseases are further reduced. Especially, the treatment of pediatric bUTIs caused by ESBL-producing *E. coli* is nowadays seriously jeopardized due to antibiotic resistance in sub-Saharan Africa countries. It should be noted that the present study has some limitations with respect to UTIs, as inclusion was restricted to febrile children only and acute UTIs can also be present in children without fever and therefore we may have missed cases. Furthermore, we have set an artificial limit to what was considered as bacteriuria and we may therefore have missed some cases.

The observed high resistance of *E. coli* to 3rd generation antibiotics cephalosporin (CRO) and fluoroquinolones (CIP), which are two essential antibiotics largely used in our study area, further pin points the severe threat of antibiotic resistance at the community level. Together these data confirm that the efficacy of many first-line antibiotics commonly used in Nanoro to treat principal bacterial infections such as *E. coli* and *NTS* is at high risk. This is likely to further undermine the precarious health system in place in low- and middle-income countries (LMICs) such as Burkina Faso if nothing is done to stop the spread of resistance. It should be noted that our results are fully in line with other observations that warned for decaying antibiotic effectiveness (17, 43). Therefore, actions have to be taken urgently to prevent the inappropriate use of antibiotics, which are still (highly) effective against common pathogenic bacteria encountered at primary health facilities. In order to deal with this threat, it is essential that practical tools or diagnostic algorithms be developed to correctly diagnose bacterial infections that can be easily implemented in the primary health care settings in LMICs. Furthermore, national and regional guidelines for integrated management of childhood illness (IMCI) that recommend syndrome-based management and treatment of bacterial infection need to be

reconsidered as it may contribute to the spread of antibiotic resistance. The untargeted, prolonged, and repeated exposure of bacteria to essential antibiotics, which is a consequence of the use of the IMCI guidelines, is largely contributing to emerging resistance and jeopardizes action plans to fight against this emerging antibiotic resistance.

Despite the rare cases of *N. meningitidis* (2 cases) and *H. influenza* b (1 case) reported in the present study, it is relevant to note that these bacteria were fully susceptible to the CL and CRO. This is important as these antibiotics are used to treat meningitis as recommended by MoH of Burkina Faso (the country is located in Lapeyssonnie's belt). Moreover, GEN used in combination with AMP as a first-line antibiotic showed to be effective against most of the pathogens isolated in this study, except for *E. coli*, which showed moderate resistance. In addition, low resistance of *NTS* isolated from blood to this antibiotic was found in the present study, and this is worrying as this combination was always highly effective against *Enterobacteriaceae* in Burkina Faso and this might be an indication for upcoming resistance against this antibiotic.

The study also reported a high prevalence of MDR bacteria. This emergence of MDR is a serious public health problem and a threat to effectively treating bacterial infections. The emergence of specific MDR bacteria is closely linked to the use of broad-spectrum antibiotics for both presumptive and definitive therapy. The occurrence of community spread of MDR bacteria leads to the large increase of the population at risk and increases the number of infections caused by MDR bacteria.

A limitation of the study is that the work did not include respiratory tract infections, as these infections are often (presumptively) treated with antibiotics, irrespective of the cause of infection (being bacterial or viral). Often this treatment practice leads to significant resistance (19, 44). In the case of suspected simple pneumonia, it is for example advised to treat with the trimethoprim-sulfamethoxazole (SXT). In the present study, it was found that this antibiotic was ineffective to many of the bacterial infections studied and it would be valuable to determine its effectiveness against bacterial infections causing pneumonia.

Another possible restriction of the study is that in some cases only few isolates could be tested for susceptibility, e.g. only 4 *S. pneumoniae*, 2 *S. aureus*, and 2 *N. meningitidis* isolates were tested in this study. According to CLSI guidelines analyzing percentage of susceptibility on isolates that are less than 30 in total number should not be done. However, we think that it is important to present the outcomes of testing on all isolates as it provides a first insight in possible evolving resistance.

The low prevalence of *S. pneumoniae* is likely a positive effect of the introduction of the pneumococcal conjugate vaccine in the expanded program of immunization (EPI) in October 2013 (45, 46). However, it remains a concern that the few isolates recovered in the present study (from blood) showed moderate to high resistance against the first-line antibiotics recommended in our study area (6, 18).

Finally, another limitation of our study is the fact that the recruited children were not followed up post-treatment in the framework of the study. Consequently, it remains unknown whether the treatments installed actually failed or were successful *in vivo*. However, based on the evidence provided by the susceptibility testing it is likely that several treatments have failed thereby jeopardizing the health of the children.

The study demonstrated that various first-line antibiotics are no longer effective to treat common bacterial infections and that alternative treatment options should be considered. Based on our study outcomes we propose that the following alternative treatments could be considered (Table 8): when sepsis or a simple (uncomplicated) bBSI is suspected; the proposed treatment would be with a single 3rd generation cephalosporin (CRO). In the case of a severe sepsis or severe bBSI, the proposed treatment would be a combination of a 3rd generation cephalosporin such as CRO combined with an aminoglycoside, like Gentamycin (GEN). In the case of a suspected bUTI, we suggest distinguishing between hospitalized and non-hospitalized cases, because the route of administration of GEN may have a health safety risk for the outpatient as it needs to be administered intravenously. For a hospitalized patient with bUTI the proposed treatment would be with an aminoglycoside (GEN). However, for a non-hospitalized case, we propose to use amoxicillin-clavulanate (AMC) which is a combination of a β-lactamase inhibitor, Clavulanic acid (C), together with another antibiotic agent, Amoxicillin, which can be administered orally. For the treatment of bGE we propose to use a fluoroquinolone (CIP), but it is important to monitor resistance to this antibiotic too as it is very frequently used even without proper laboratory examinations and/or prescriptions.

Table 8
Proposed alternative antibiotic treatments to treat common bacterial infections.

| Infection type | Proposed alternative antibiotic to be used based on the study outcome |
|---|---|
| Suspicion of a simple bBSI | CRO |
| Suspicion of a serious bBSI | CRO + GEN |
| bUTI in a hospitalized patient | GEN |
| bUTI in a non-hospitalized patient | AMC |
| bGE | CIP |

*bBSI: bacterial bloodstream infections (blood stream infections and meningitis); bGE: bacterial gastroenteritis; bUTI: bacterial urinary infection; CRO: ceftriaxone; GEN: gentamycin; AMC: amoxicillin-clavulanate; CIP: ciprofloxacin.*

## Conclusions

In conclusion, this study showed an alarming high resistance to many first-line antibiotics used to treat common bacterial infections in Burkina Faso. The work prompts the need to expand antibiotic resistance surveillance studies in Burkina Faso, and probably the whole region (West Africa).

## Abbreviations

ABR: antibiotic resistance; AMOX:amoxicillin; AMP:ampicillin; AMR:antimicrobial resistance; API:analytical profile; AST:antimicrobial susceptibility testing; ATCC:American type culture collection; bBSI:bacterial bloodstream infection; bGE:bacterial gastroenteritis; bUTI:bacterial urinary tract infection; CAZ:ceftazidime; CAZ + C:ceftazidime + clavulanate; CC:clindamycin; CFU:colonies forming units; CIP:ciprofloxacin; CL:chloramphenicol; CLED:cysteine-lactose-electrolyte-deficient; CLSI:clinical and laboratory standards institute; CRO:ceftriaxone; CRUN:clinical research unit of Nanoro; CTX:cefotaxime; CTX + C:cefotaxime + clavulanate; EMB:eosin methylene blue; EPEC:enteropathogenic *E. coli;* EPI:extended program of immunization; ERY:erythromycin; ESBL:extended ß-lactamase; FOX cefoxitin; GEN:gentamycin; IMCI:integrated management of childhood illness; IVX:isovitalex; LMICs:low-and-middle- income countries; MDR:multidrug resistance; MH:Mueller Hinton; MIC:minimal inhibitory concentration; MoH:ministry of health; MRSA:methicillin-resistant *Staphylococcus aureus*; NA:nalidixic acid; *NTS*:*non-thyphoidal Salmonella*; NICD:national institute for communicate disease; PEN penicillin; PVX:polyvitex; SBA:sheep blood agar; SOP:standard operating procedure; SS:*Salmonella-Shigella*; SXT:trimethoprim-sulfamethoxazole; TET:tetracycline; *TS*:*thyphoidal Salmonella*; WHO:World Health Organization.

## Declarations

### *Ethics approval and consent to participate*

Written informed consent was obtained from a parent or legal guardian of each child prior to enrolment in the study. The study protocol was reviewed and approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N (2014-11-130).

### *Consent for publication*

Not applicable.

### *Availability of data and materials*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### *Competing interests*

The authors declare that they have no competing interests.


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### *Authors' contributions*

This study was conceived and designed by MB, MT, FK, HS, PM, HT and SM. Recruitment of children and various clinical specimen's collection was supervised by MB and AS, the latter also being responsible for supervision of medical care. Microbiology analyses and resistance profiling was performed MB, FK, MT, SY, and PL. The manuscript was drafted by MB, FK and HS and reviewed by all authors, who also approved the final version of manuscript.


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The American Type Culture Collection (ATCC®) provided standard reference strains *Escherichia coli* ATCC® 25922 $^{TM}$, *Salmonella thyphimurium ATCC*® 14028$^{TM}$, *Staphylococcus aureus* ATCC® 25923$^{TM}$, *Staphylococcus epidermidis* ATCC® 14990 $^{TM}$, *Streptococcus pyogenes* ATCC® 19615 $^{TM}$, *Enterococcus faecalis* ATCC® 29212 $^{TM}$, and *Streptococcus pneumonia* ATCC® 49619 $^{TM}$ to the CRUN laboratory.


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