Research Article
Open Access
CrossMark

Multirésistance to antibiotics of pathogens isolated from human, animal and environment in the District of Bamako, Mali

Abstract

In Mali, *E. coli* and *Salmonella* infections are one of the most common bacterial diseases in outpatients. Treatment of these diseases has become challenging due to the emergence of pathogens with increasing resistance to available antimicrobial agents. The aim of this study is to determine the prevalence and evaluate the antibiotic resistance profile of the most frequently isolated strains of these bacteria involved in bacterial infections in Bamako, Mali. In this study, 400 samples of human, animal and environmental origin were obtained in the study area. The isolated bacteria were identified by biochemical tests. Disk diffusion method was applied to determine the antibiotic sensitivity of bacterial agents. Our results showed that, only 321 enterobacteria were isolated from the 958 samples analyzed. The most isolated pathogenic bacteria was *E. coli* with frequency rate of 20.77%. The others enterobacteria were *Klebsiella* sp. (6.05%), and *Salmonella* sp. (3.86%). All pathogenic bacteria isolated in this study, were highly resistant to Amoxicillin, Ticarcillin (100% for *Klebsiella* sp., isolated from all samples), and sensitive to Imipenem, Cefoxitine and Nitrofurantoine. According to the present survey, apart from *Salmonella* isolated from human and environmental samples, all the pathogenic bacteria isolated from human, animal and environmental sources showed multiple antibiotic resistance with a MAR index as high as 0.7 for *E. coli* from human source. This high level of multiresistance indicates that these human, animal and environment at Bamako could constitute a "high risk" source of antibiotic contamination.

Keywords: Africa, bacteria, disk diffusion, MAR index, one health

Volume 8 Issue 2 - 2023

Judicaël Ouédraogo,[2] Lassina Gadi Timbiné,[2] Bréhima Traoré,[2] Abdoul Karim Sangaré,[2] Elisabeth Sogodogo,[2] Kaisa Haukka,[2] Bourèma Kouriba,[2] Amadou Hamadoun Babana[1*]

[1]LaboREM-Biotech, FST, USTTB, Mali
[2]Department of Biology, Laboratoire Merieux, Mali

***Correspondence:** Amadou Hamadoun Babana, Department of Biology, USTTB, Mali, Tel +22376124173, Email ahbabana@laborem-biotech.com

Received: April 09, 2023 | **Published:** April 20, 2023

Introduction

The One Health approach aims to attain optimal health for people, animals and the environment.[1,2] Antibiotic resistance (ABR) is recognized as a One Health challenge because of the rapid emergence and dissemination of resistant bacteria and genes among humans, animals and the environment at a global scale.[3] Antibiotic resistance has disproportionately affected developing countries, making this issue a significant development challenge. In Sub-Saharan Africa, this problem is aggravated by other factors such as the illicit sale of antibiotics, self-medication, poor hygienic conditions in the hospitals and the lack of knowledge in the fight against antibiotic resistance. For example, in parts of Asia and Africa, an unprecedented epidemic of typhoid fever had spread due to a multidrug-resistant clone of the bacteria, known as H58.[4–6] Similarly, in the province of Sindh in Pakistan, another epidemic also occurred between 2016 and 2018, from which 5274 people were affected by typhoid fever with high resistance to antibiotics.[7] This urgent threat was recognized by the World Health Organization's 2015 Global Action Plan.[8] the 2016 United Nations General Assembly's unanimous commitment to combat antimicrobial resistance (AMR).[9] and the Association of Southeast Asian Nations 2017 Statement on AMR.[10] AMR, a serious threat to the provision of safe and effective medical care, has the potential to impact health as much as unexpected outbreaks of high fatality diseases, albeit more slowly but also with more certainty.[11] In some regions, the increased resistance has been so widespread that some bacteria show resistance to almost all of these drugs.[12] This dissemination concerns not only the bacteria themselves, but also the resistance genes they carry and which can be acquired by other bacteria.[12] Considering the high recurrence rates and emergence of antibiotic resistance in human, animal and environment, knowledge about the source-specific prevalence of these pathogens and their multi antimicrobial resistance indexes, is important. Also, in order to select more appropriate antibiotics and prevent therapeutic failures, it is important to determine pathogens and their antibacterial susceptibility patterns. That is why the aims of this study were identification of pathogenic agents in human, animal and environmental samples and determination of their antibiotics resistance.

Material and methods

Study site and sample collection

This study focused mainly on biological samples, the sampling of which was carried out across the district of Bamako from 2019 to 2022. During these 3 years, four hundred (400) samples of human (628 samples), animal (132 samples) and environmental origin (198 samples) were taken in the study area (Figure 1).

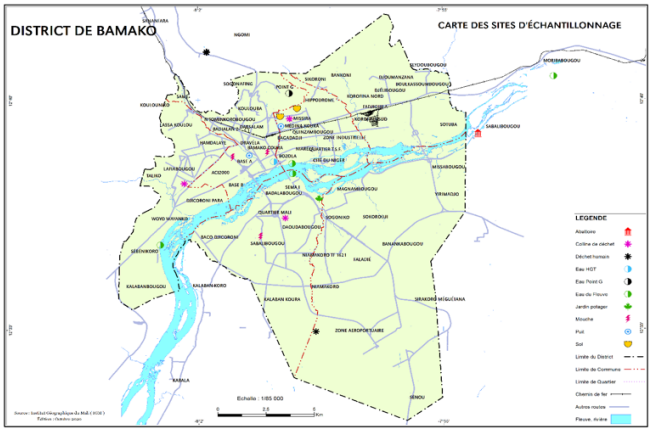

Figure 1 Map of study area and sampling sites (District of Bamako, Mali).

These are samples of soil contaminated by human waste, vegetable gardens, waste water (from hospitals, pipelines, the Niger River and refrigerated slaughterhouses in Bamako), untreated drinking water (wells), faeces of animals and flies, fish viscera and human samples intended for routine microbiological analyzes received at the LRM within the Center of Infectiology of Charles Mérieux du Mali (CICM-Mali) located in Bamako during the study period. The samples of human origin were taken according to the sites of infection depending on whether they are samples of urine, pus, sputum, blood cultures, puncture fluids received at the LRM. Wastewater samples were collected in sterile 2500 ml polyethylene pots (Ref. 77.580), while soil samples, animal faeces and fish intestines were received in sterile plastic bags of the "Biohazard Specimen Bag" type (Ref. 8078193855). The collected samples were protected from direct sunlight and transported in a cooler box containing ice packs to the laboratory for analyses. All samples were stored at 4°C and analyzed within 24 hrs of sample collection.

Bacterial isolation

The water samples were analyzed for the target bacteria using the standard methods for the examination of water and wastewater.[13] According to[13] prior to analysis, the water samples were mixed to distribute bacteria uniformly. Then, dilutions of water samples from 10^{-2} to 10^{-6}, were prepared. Fifty milliliters from each dilution replicate of each water sample was filtered using a 0.45 μm diameter, cellulosic grid filter placed on the filter holder. To wet the filter paper, approximately 25 ml of distilled water was first added. Bacteriological analysis of samples from soils and animals were done according to.[14,15] In human samples, pathogen bacteria were isolated according to methods decribed by.[16] Selective media for each pathogenic bacteria (Table 1) were used and prepared according to the procedure recommended by the manufacturer and sterilized by autoclaving at 121°C for 15 minutes. Treated membrane filters and dilutions of animal and human samples were aseptically transferred to 90 mm Petri dishes with the appropriate selective media.

Multi drug resistant (MDR) testing for isolated bacteria

In this study, the antimicrobial susceptibility test was done on Mueller-Hinton agar (Merck, Germany) using the disk diffusion (Kirby Bauer's) technique.[17] following disk diffusion methods in the Manual of Antimicrobial Susceptibility Testing. The disks of antibiotic used comprised: Amoxicilline 25 µg; Amoxicilline + Acide clavulanique 20/10 µg; Tobramycine 10 µg; Gentamycine 15 µg; Ciprofloxacine 5 µg ; Ticarcilline 75 µg; Cefoxitine 30 µg ; Ceftazidime 30 µg; Cefotaxine 30 µg; Acide Nalidixique 30 µg; Amikacine 30 µg; Imipenem 10 µg; Nitrofurantoine 300 µg ; Fosfomycine 50 µg; Sulfamethoxazole + triméthoprime 1.25/23.75 µg; Norfloxacine 5 µg; Pefloxacine 5 µg. The test of antibacterial resistance of all strains of bacteria was performed using the Kirby- Bauer disc diffusion method. Briefly, each pure overnight bacterial culture was suspended in physiological saline to have a standard turbidity of 0.5 McFarland, that was streaked on Mueller Hinton agar platesand incubated 37°C for 24 hours. After overnight incubation, the inhibition zone around each disc was measured based on the interpretive standard of the Clinical and Laboratory Standards Institute (CLSI) used to define the bacterial isolates as sensitive, intermediate or resistant to the antibiotic evaluated.

Multiple antibiotic resistance index

For each pathogenic bacterial isolate, a multiple antibiotic resistance (MAR) index was determined by using the formula MAR = a/b, where a is the number of antibiotics to which the test isolate depicted resistance and b the total number of antibiotics to which the test isolate has been evaluated for susceptibility.[18] A high-risk, where antibiotics are often used, is indicated by a MAR value of 0.2 and higher.

Results

Bacterial strains isolated from environmental, human and animal samples

A total of 321 enterobacterial colonies were isolated in 958 analyzed samples from the environment, humans and animals in Bamako and surroundings (Table 1). The total enterobacterial colonies isolated, which depend on the sources, are distributed as follows: 60 colonies isolated from environmental samples, 202 colonies were isolated from human samples, and 59 colonies were isolated from animal samples (Table 1). From the 321 enterobacteria isolated, 199 were *Escherichia coli,* 85 *Klebsiella* spp., and 37 *Salmonella* sp.

Antibiotic succeptibility among isolated bacteria

Among all the beta-lactams tested, amoxicilline and Tircacilline were the least active antibiotics in samples from humans and the environment (Table 2). Imipeneme and Cefoxitine were active against all strains of *E. coli* isolated. Tobramycin was the least active aminoside. The strains of *E. coli* isolated from the human sector were more resistant to quinolones than those isolated from the other two sectors. Among all the other antibiotic molecules tested, Sulfamethoxazole-Trimethoprim was the least active antibiotic. Fosfomycine and nitrofurantoin showed less efficacy in the environment. All strains of *Klebsiella* sp., were resistant to amoxicillin (100%) and ticarcilline (100%). Resistance to beta-lactams was higher in humans than in animal samples. A low resistance to imipenem was observed among strains of *Klebsiella* sp. isolated only from humans, unlike other sectors where all strains were susceptible. *Salmonella* strains isolated in animal samples exceeded the limit 0.2 of MAR index, whereas *Salmonella* sp. strains isolated in human and environmental samples showed no or little resistance to all the tested antibiotics.

Multi-antibiotics resistance of isolated bacteria

The susceptibility of tested bacterial pathogens to antibiotics (Table 2) showed that all studied bacterial strains were multidrug resistant, with a MAR index from 0.24 to 0.73, with exception of *Salmonella sp.,* isolated from human and environmental samples.

Table 1 Number of total bacterial colonies of *E. coli, Klebsiella spp.* and *Salmonella sp.* isolated from analyzed human, animal and environmental samples

	Number of isolated enterobacteria per source			
Bacteria	**Humans**	**Animals**	**Environment**	**Total**
E. coli	129	40	30	199
Klebsiella spp.	48	11	26	85
Salmonella sp.	25	8	4	37

Table 2 Antibiotic resistance among bacterial isolates (%)

Antibiotics	Humans			Animals			Environment		
	E. coli[c]	*Kle*[b]	*Sal*[a]	*E. coli*	*Kle*	*Sal*	*E. coli*	*Kle*	*Sal*
Amoxicilline	85.27	100	4	47.5	100	25	73.33	100	0
Amoxicilline-Acide clavulanique	57.36	60.42	4	7.5	0	25	43.33	42.31	0
Imipenem	0	4.17	0	0	0	0	0	0	0
Cefotaxime	46.51	52.08	0	0	0	25	20	19.23	0
Ceftazidime	45.74	47.92	0	2.5	0	25	6.67	15.38	0
Ticarcilline	85.27	100	0	47.5	100	25	46.67	100	0
Cefoxitine	0	4.17	0	0	0	0	0	23.08	0
Tobramycine	42.64	37.5	0	47.5	9.09	0	63.33	30.77	0
Amikacine	6.21	20.83	0	32.5	0	0	23.33	3.85	0
Gentamycine	31.78	29.17	0	10	9.09	0	10	7.69	0
Acide-Nalidixique	64.34	20.83	0	25	9.09	50	43.34	11.54	25
Ciprofloxacine	56.59	43.75	4	12.5	18.18	50	26.66	15.38	25
Norfloxacine	53.49	45.83	0	30	0	50	33.33	11.54	0
Fosfomycine	0	45.83	0	0	36.36	12.5	6.67	11.54	25
Nitrofurantoine	0	16.67	4	0	0	0	6.67	15.38	25
Sulfamethoxazole-Trimethoprime	87.6	62.5	56	47.5	54.55	75	36.67	11.54	50
MAR index	0.73	0.68	0.05	0.24	0.27	0.29	0.37	0.38	0.1

[a]*Salmonnella* sp., [b]*Klebselia* sp.,[c]*Escherichia coli*

Discussion

All pathogenic bacteria isolated from human, animal and environmental samples from Bamako and surroundings, and tested against all the antibiotics used; showed resistance to at least five antibiotics. The municipal and industrial wastewater discharge into the Niger river water in Bamako can favorize the presence of residual antibiotics and antibiotic contamination in the river water.[19,20] This can cause the emergence and development of bacteria resistant to antibiotics in the river water. Some studies.[21] indicate that the accumulation of municipal sewage with fecal wastes seems to be mainly responsible for deterioration of river water quality along with increased population of pathogenic bacteria resistant to antibiotics in the river.

All pathogenic bacteria isolated in this study, were highly resistant to Amoxicillin, Ticarcillin (100% for *Klebsiella* sp., isolated from all samples, with the exception of *Salmonella* sp. in environmental and human samples) and Sulfamethoxazole-Trimethoprime (62.5% and 54.5% of resistance, respectively for *Klebsiella sp.* from human and animal sources). Urban agriculture, hotels and livestock can become the cause of environmental pollution by antibiotics and emergence of antibiotic resistant bacteria, and the increase of pathogenic bacteria resistant to antibiotics may affect negatively and seriously the public health and environment.[22–24]

All *E. coli* and *Klebsiella* sp. isolates were highly resistant to at least 5 tested antibiotics. Authors of the review [25] on antibiotic resistance patterns in human, animal, food and environmental isolates in Ghana reported that the highest mean resistance rate was encountered in *Escherichia coli* (62.2%) followed by *Klebsiella* spp. (60.4%). Kumar et al.[26] who evaluate the occurrence of bacteria resistant to antibiotics, the antibiotic-resistant gene, and the concentration of metal in the river of Sri Lanka, show that: for older antibiotics, the percentage of resistance of *E. coli* is higher than for other antibiotics. Among all the beta-lactams tested, amoxicillin was the least active antibiotic with resistance rates of 85.27% in humans, 47.50% in animals and 73.33% in the environment. At the same time, all the isolated bacteria showed no or small resistance to Imipenem, Cefoxitine and Nitrofurantoine. Our results showed that Imipenem and Cefoxitin were active against all strains of *E. coli* isolated. The low or absence of resistance of the isolated bacteria, mainly pathogenic *E. coli*, to Imipeneme and Cefoxitine may indicate that all the sample sources (human, animal and environment) has been contaminated with low levels of these antibiotics, and indicates low use of Imipenem and Cefoxitine in human activity in Bamako. Tobramycin was the least active aminoside with resistance rates of 42.64% in humans, 47.50% in animals and 63.33% in the environment. Talon et al.[27] working on the resistance to quinolones and β-lactams of clinical *E. coli* strains isolated in the Franche-Comté region of France showed that multi-drug resistance was highly prevalent in *E. coli* and that these *E. coli* isolates showed high resistant rate (>80%) to several old drugs, including ampicillin, tetracycline and sulfisoxazole. This study also showed that *E. coli* strains isolated from human samples were more resistant to quinolones than those isolated from animal and environmental sources. Quinolones are the antimicrobial agents of choice for treatment of various infections caused by *E. coli* or other Gram-negative bacteria. However, because of extensive use for multiple clinical indications in human or veterinary medicine, bacterial resistance to quinolones has developed over the time.[27]

In this study, a total of 321 pathogenic bacteria; out of which (199 *E. coli*, 85 *Klebsellia* and 37 *Salmonella*; isolated from human, animal and environmental samples are resistant to, at least, five antibiotics and have a MAR index greater than 0.2 indicating a "high risk" human source of antibiotic contamination. Similarly, all the pathogenic *E. coli* and *Klebsiella* sp. isolated in human, animal and environmental samples have a MAR index greater than 0.2. Koumaré et al.[28] isolating antibiotic resistant bacteria from river water in Bamako, Mali, obtained 174 bacteria out of which 142 (81.4%) were resistant to at least two antibiotics. Those results show that the Niger river is highly polluted. Meanwhile, for *E. coli* and *Klebsiella* sp. isolated from human samples, showed a MAR index of 0.7 and 0.68 respectively. Based on these results, the bacterial pathogens isolated from human sources were highly multi-drug resistant. In human pathogenic bacteria, the high prevalence of resistance to the commonly used antibiotics such as ampicilllin, cephalothin and tetracycline has caused considerable

alarm.[29, 30] The Niger river, an environmental source of resistant bacteria, is located in the urban area which is affected by residential area, the industrial and agricultural activities, and small gardens along the river.[31] In this study, samples from the Niger river water in Bamako were taken near the martyrs' bridge between the Marietou Palace Hotel and the Kampesky Hotel. hese results clearly show the emergence of antibiotic-resistant bacteria at the human, animal and environmental sampling sources.

Conclusion

A total of 958 pure colonies of bacteria were successfully isolated in the human, animal and environmental samples from Bamako district and its surrondings. Among these bacteria; 199 were *Escherichia coli,* 85 *Klebsiella* spp., and 37 *Salmonella* sp. Apart from strains of *Salmonella* sp., isolated from human and environmental samples, all the pathogenic bacteria isolated from human, animal and environmental samples tested have a MAR index greater than 0.2. *E. coli* and *Klebsiella* spp. from human samples had MAR index values 0.73 and 0.68 respectively. The results obtained showed that most of the pathogenic bacteria isolated from human, animal and environmental sources in Bamako showed multiple antibiotic resistance, indicating that these human, animal and environment at Bamako could constitute a "high risk" source of antibiotic contamination.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

References

1. King LJ, Larry RA, Carina GB, et al. Executive summary of the AVMA one health initiative task force. *J Am Vet Med Asso.* 2008;233:259–261.
2. Rousham EK, Unicomb L, Islam MA. Human, animal and environmental contributors to antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and one health approaches. *Proc R Sci*. 2018;285(1876):20180332.
3. Robinson TP, Carrique M J, Fèvre EM, et al. Antibiotic resistance is the quintessential one health issue. *Trans R Soc Trop Med Hyg*. 2016;110(7):377–380.
4. World Health Organization (WHO). *Global action plan on antimicrobial resistance*. WHO: Geneva, Switzerland; 2015.
5. Aarestrup FM, Wegener HC, Collignon P. Resistance to bacteria in the food chain: epidemiology and control strategies. *Expert Rev Anti Infect Ther*. 2008;6(5):733–750.
6. Jamilah J, Hatta, M , Natzir R, et al. Analysis of existence of multidrug-resistant H58 gene in Salmonella enterica serovar Typhi isolated from typhoid fever patients in Makassar, Indonesia. *New Microbe and New Infect*. 2020;38:100793.
7. *Dall C.* WHO:XDR typhoid outbreak in Pakistan tops 5,200 cases. *In CIDRAP New*; 2018.
8. O Neill J. *Tackling drug resistant infections globally: final report and recommendations*. London; Wellcome Trust and Government of the United Kingdom; 2016.
9. World Bank. *Drug resistant infections: a threat to our economic future*. World Bank, Washington, DC; 2017.
10. Reinl J. UN declaration on antimicrobial resistance lacks targets. *Lancette. 2016;*388(10052):1365.
11. Torren EJ, Grave K, Mackay D. One health: the regulation and consumption of antimicrobials for animal use in the EU. *IHAJ.* 2015;2:14–16.
12. Robinson TP, Bu DP, Carrique MJ, et al. Antibiotic resistance is the quintessential one health issue. *Trans R Soc Trop Med Hyg.* 2016;110(7):377–380.
13. 13 APHA. *Standard Methods for the Examination of Water and Wastewater.* American Public Health Association, Washington, DC, USA ; 2017.
14. Babana AH, Samaké F, Maïga K. Characterization of some agricultural soils: presence and activity of tilemsi rock phosphate solubilizing thiobacilli. *British Microbiology Research Journal*. 2011;1(1):1–9.
15. Dicko AH, Babana AH, Maïga K, et al. Isolation and characterization of crop rhizospheric actinomycetes with antimicrobial activity in Mali. *African Journal of Applied Microbiology Research*. 2013;2(1):1–8.
16. Kanadjigui M, Babana AH, Traoré B, et al. Prevalence and antibiotic resistance profile of enterobacteria involved in urinary infections in bamako, Mali. *MOJ Biol Med*. 2017;1(4):1–4.
17. Ortez, J. *In Manual of Antimicrobial Susceptibility Testing*. American Society for Microbiology: Washington, DC, USA; 2005. 39–52 p.
18. Salikan NA, YernKK, Zain NA. Isolation of antibiotic resistant bacteria from rivers in terengganu, malaysia. *International Journal of Life Sciences and Biotechnology*. 2020;3(2):241–257.
19. Aslam B, Wang W, Arshad MI, et al. Antibiotic resistance: a rundown of a global crisis. *Infecter. Résistance à la drogue.* 2018;11:1645.
20. Woolhouse MEJ, Ward MJ. Sources of antimicrobial resistance. *Science*. 2013;341(6153):1460–1461.
21. Heuer OE, Kruse H, Grave K, et al. Human health consequences of use of antimicrobial agents in aquaculture. *Clin Infect Dis.* 2009;49(8):1248–1253.
22. Hernando AS, Coque TM, Baquero F, et al.. Defining and combating antibiotic resistance from the point of view of one health et global health. *Nat Microbiol.* 2019;4(9):1432–1442.
23. O Neill J. Antimicrobials in agriculture and the environment: reducing unnecessary use and waste. *The Review on Antimicrobial Resistance*; 2015.
24. Shah TA, Roach S, Ling CY, et al. An integrated systems approach is needed to ensure the sustainability of antibiotic effectiveness for both humans and animals. *J Law Med Ethics.* 2015;43:38–45.
25. Gales AC, Tognim MCB, Munerato P, et al. Quinolone resistant *Escherichia coli*. *Braz J Infect Dis*. 2008;12(1):5–9.
26. Kumar M, Chaminda GGT, Honda R. Seasonality impels the antibiotic resistance in kelani river of the emerging economy of sri lanka. *NPJ Clean Water*. 2020;3:12.
27. Talon D, Thouverez M, Bertrand X. *Escherichia coli* : résistance aux quinolones et aux β-lactamines des souches cliniques isolées en franche-comté [*escherichia coli*: resistance to quinolones and β-lactams of clinical strains isolated in the franche-comté region of france]. *Pathologie Biologie*. 2004;52(2): 76–81.
28. Koumaré Y, Babana AH, Bah A, et al. Bacteria isolated from niger river water in bamako showed multi-resistance to antibiotics. *MOJ Biol Med*. 2022;7(2):71–74.
29. Olaitan JO. Asymptomatic bacteriuria in female students population of a nigerian university. *Internet J Microbiol.* 2006;2(2):2–6.
30. Orrett FA, Davis GK. A comparison of antimicrobial susceptibility profile of urinary pathogens for the years; 1999 and 2003. *West Indian Med J*. 2006;55(2):95–99.
31. Amalina NZ, Santha S, Zulperi D, et al. Prevalence, antimicrobial susceptibility and plasmid profiling of *vibrio* spp. Isolated from cultured groupers in peninsular malaysia. *BMC Microbiology*. 2019;19:251.