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Full Length Research Paper

Multidrug resistant bacteria isolated from nosocomial infections at University Teaching Hospital of Point-G, Bamako, Mali

Maiga Aminata[1*], Beye Seydina Alioune[2,3], Cissoko Yacouba[2,4], Dicko Oumar Agaly[1], Diarra Bassirou[2,5], Traoré Abdoulaye[7], Coulibaly Djibril Mamadou[1,6], Koné Drissa[1], Diarra Lobogal[1], Coulibaly Djaminatou[1], Coulibaly Youssouf[2,3], Maiga Ibrahim Izetiégouma[1,2] and Fofana Djeneba Bocar[2,5]

[1]Medical Biology and Hospital Hygiene Laboratory, Point-G University Hospital, Bamako, Mali.
[2]Faculty of Medicine and Odontostomatology, University Teaching Hospital of Point-G, Bamako, Mali.
[3]Point-G University Hospital Resuscitation Unit, Bamako, Mali.
[4]Department of Infectious and Tropical Diseases, Point-G University Hospital, Bamako, Mali.
[5]University Center for Clinical Research, Point-G University Hospital, Bamako, Mali.
[6]Faculty of Pharmacy, Point-G University Hospital, Bamako, Mali.
[7]National Institute for Training in Health Sciences, Mali.

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An infection is said to be nosocomial or hospital if it is absent when the patient enters the hospital and it appears and develops at least 48 h late. The objective of this study was to determine the resistance phenotypes of bacteria isolated from nosocomial infections at the University Teaching Hospital of Point G. Urine, blood, pus, skin and bronchoalveolar fluid samples were taken in different units, and bacteria isolations were performed on usual selective media such as Drigalski Colombia agar supplemented with nalidixic acid and colistin and 5% sheep blood and chocolate agar. Identifications of bacteria such as *Enterobacteriaceae, Pseudomonas and acinetobacter, and* Staphylococci were done using API20^{E} gallery, API20^{NE} gallery and catalase/oxidase tests, and the Pastorex Staph kit respectively. The antimicrobial susceptibility testing was performed on Mueller-Hinton agar using the diffusion method. A total of 463 patients were inpatients for at least 48 h in the different units, and a nosocomial infection was notified in at least 57 patients (12.3%). A total of 65 episodes of nosocomial infections were observed in these 57 patients. Of the bacteria isolated, multidrug-resistant bacteria (MDR) represented 63.7% (n=36). These were extended-spectrum beta-lactamase (ESBL)-secreting *Enterobacteriaceae* (n=21), high-level cephalosporinase (n=13) and methicillin-resistant coagulase-negative Staphylococci (n=2). Despite this high number of multi-resistant bacteria isolated in this study; colistin and amikacin had very good activity on *enterobacteriaceae.* The results show the need to strengthen hygiene in the intensive care units in order to fight against nosocomial infections at the UTH of Point G.

Key words: Nosocomial infections, multi-resistant bacteria, UTH of Point-G.

INTRODUCTION

An infection is said to be nosocomial or hospital if it is absent when the patient enters the hospital and it appears and develops at least 48 h late. According to the World Health Organization (WHO), more than 1.4 million people worldwide suffer from hospital-acquired infections (OMS, 2017). In the United States, the risk of being a

victim of this type of infection has increased steadily over the past decades and resulted in expenditure estimated at $4.5 to 5.7 billion per year (Statistiques, 2017). In France, 6 to 7% of hospitalizations are complicated by a more or less serious NI. The rates of healthcare-associated infections (HAIs) and bacterial resistance in developing countries are 3 to 5 times higher than international standards. In countries with limited resources, such as West African nations, other features, more specifically socioeconomic and behavioral factors, contribute to exacerbate this problem (Ouedraogo et al., 2017). The nosocomial infections fall within a range of colossal costs going from around 340 euros for a urinary tract infection to 4000 euros for an infection contracted in the intensive care unit (Boulahouat and Aliziane, 2019). HAIs can extend the length of stay to 10 days, increase costs (US$5,000 to US$12,000) and mortality (by a factor of 2 to 3) (Rosenthal, 2008; Boulahouat and Aliziane, 2019).

Resource-limited countries have higher rates of device-associated healthcare-associated infections (HAIs), including central line-associated bloodstream infection (CLAB), ventilator-associated pneumonia (VAP), and infection Catheter-Associated Urinary Tract (CAUTI), 3 to 5 times higher than rates reported in intensive care units in North America, Western Europe and Australia (Rosenthal, 2008). Africa has the highest rate estimated at 25% (Kakupa et al., 2016). In Côte d'Ivoire, the emergence of multidrud-resistant bacteria (MDR) responsible for infections in hospitals has been observed in recent years (Guessend, 2008; Méité et al., 2009) and in Senegal, global incidence multi-resistant bacteria was 5.5% with an incidence density of 5 cases per 1000 patient-days at the University Hospital of Fann in Dakar (Fortes et al., 2015).

In Mali, several studies have been carried out to determine the extent of NI, and different prevalence rates varying was found from 4.72 to 29. 4% (Dembélé, 2015; Dembele, 2017; Beye et al., 2022; Bocoum et al., 2020; Dicko et al., 2022).

Moreover, a study conducted in 2002 in five hospitals in Bamako showed a prevalence of NI at 14.4% (Maiga, 2002) Thus, the objective of this study was to determine the resistance phenotypes of bacteria isolated from nosocomial infections at the University teaching Hospital of Point G.

METHODS

Study design and settings

This was a descriptive and analytical cross-sectional prospective study over a period of 6 weeks from 1st July to 18th August, 2019 which was carried out in 10 departments of the University Teaching Hospital (UTH) of Point G including the Intensive Care Unit, general surgery A and B, gynecology-obstetrics, neurology, nephrology and hemodialysis, rheumatology, internal medicine, infectious and tropical diseases; and urology. The University Teaching Hospital of the Point G is the third-pyramidal' reference in Mali, and has 522 beds divided between the surgical, intensive care and medical departments.

All hospitalized patients were included in this study for at least 48 h with a suspicion of infection or confirmed by the presence of at least two criteria of the systemic inflammatory response syndrome (SIRS) such as a temperature >38.5°C or < 36°C, heart rate > 90 beats per minute, respiratory rate > 20 cycles/minute or $PaCO_2$ < 32 mm Hg, white blood cells > 12000/mm^3 or < 4000/mm^3 or > 10% of immature forms, observed after at least 48 hours of hospitalization, and which were neither present nor incubating at the time of hospitalization.

Sample types and patients

The samples were blood cultures which were taken by nurses or interns at the time of fever peaks (temperature ≥ 38.5°C) or in the event of hypothermia (temperature < 36°C) by puncture of a non-perfused vein in two different bottles (aerobic, anaerobic). The cyto-bacteriological examinations were carried out on the first urines in the morning, or after clamped the urinary catheter for about 20 minutes. The suppuration samples were collected under strict aseptic conditions using a swab moistened with sterile 0.9% saline solution, and Broncho alveolar lavage (BAL) was performed by a pulmonologist using a bronchoscope after instillation of 100 mL of sterile isotonic saline through the trachea, before the secretions were aspirated and collected in a sterile bottle. All samples were taken according to the site of infection and sent directly to the laboratory for further investigation.

Laboratory procedures

Isolation of bacterial strains

Bacterial cultures were carried out on the usual selective media such as Drigalski (Bio-Rad, France) Colombia agar supplemented with nalidixic acid and colistin and 5% sheep blood and chocolate agar (Bio-Mérieux, France). While Enterobacteriaceae were identified using the API20^E gallery (Bio-Mérieux, France), *Pseudomonas* and *Acinetobacter* were identified using the combination of catalase/ Oxidase (Bio-Rad, France), and the API 20^{NE} gallery (Bio-Mérieux, France). *Staphylococcus* was identified using the Pastorex Staph kit (Bio-Rad, France).

Antimicrobial susceptibility testing and reading

The antibiogram was carried out on Mueller-Hinton agar (Bio-Rad, France) using the diffusion technique in agar medium. After inoculation and drying of the Mueller-Hinton agars, the discs of blotting paper impregnated with the following antibiotics were tested for Gram-negative bacilli: ticarcillin (30µg), nalidixic acid (30µg), ciprofloxacin (5µg), cefotaxime (30µg), amoxicillin + clavulanic acid (20/10µg), ceftazidime (30µg), cefoxitin (30µg), imipenem (10µg),

*Corresponding author. E-mail: mimimaiga@yahoo.fr. Tel: +223 75 89 80 54

chloramphenicol (30µg), tetracycline (30µg), gentamicin (15µg), amikacin (30µg),sulfamethoxazole (30µg), trimethoprim (30µg), colistin (30µg), by depositing them on the surface of the inoculated dishes using a disc dispenser (Bio -Rad, France). All strains were systematically detected for the production of an extended-spectrum beta-lactamase by the synergy test. This test consists of adding to the disc of amoxicillin associated with clavulanic acid and those of third generation cephalosporins. Then, after incubation at 37°C for 18 to 24 h, the production of broad-spectrum beta-lactamase results in the appearance of an image of synergy or champagne cork between the discs. Before being used, an internal control of the antibiotic discs was carried out using the reference strain of *Escherichia coli* ATCC 25922 to ensure the validity of the results obtained (CA-SFM, 2019). The antibiotic discs for which the inhibition diameters obtained are within the interval of the critical diameters present in the reading chart of the CA-SFM, 2019 were considered to be compliant.

Similarly, the blotting paper discs impregnated with the following antibiotics were tested on the strains of Staphylococci: penicillin G (10UI), amoxicillin (10µg), cefoxitin (30µg), oxacillin (5µg), chloramphenicol (30µg), kanamycin (30 IU), gentamicin (15µg), tobramycin (10µg), netilmicin (30µg), amikacin (30µg), tetracycline (30µg), ciprofloxacin (5µg), fusidic acid (30µg), fosfomycin (50µg), sulfamethoxazole (30µg), trimethoprim (5µg), erythromycin (15µg), lincomicin (15µg), pristinamycin (15µg).

In addition, strains of Enterobacteriaceae and *Acinetobacter* resistant to amoxicillin (30µg), ticarcillin (30µg), amoxicillin+clavulanic acid (20/10µg), cefalotin (30µg), cefoxitin (30µg), ceftazidime (30µg) and cefotaxime (30µg) but sensitive to imipenem (10µg) were considered high-level cephalosporinase producers (Courvalin and Philippon, 1989).

Enterobacteriaceae strains that have a positive synergy test between amoxicillin + clavulanic acid and 3rd generation cephalosporins (cefotaxime and ceftazidime) were considered as extended-spectrum beta-lactamase producers (Bonnet, 2012). Moreover, the strains of coagulase-negative Staphylococci resistant to cefoxitin and oxacillin were considered resistant to methicillin (Carttoir and Leclerq, 2012).

The readings of the results were performed by measuring the diameters of inhibition using a caliper or a graduated ruler. The results were transcribed into sensitive (S), intermediate (I), or resistant (R) categories according to the recommendations of the CA-SFM-EUCAST, 2019 and the different phenotypes of the strains were determined according to the families of antibiotics tested.

Statistical and data analysis

The data were collected on a pre-established form from admission and treatment registers, temperature sheets, medical records, surgery report registers, intensive care unit reporting sheets, results of bacteriological tests. A delegate has been appointed to inform the investigator in the event of suspected infection in each department concerned.

Data were entered and then analyzed using SPSS version 22 software. Quantitative variables were expressed as the mean (± standard deviation). Qualitative variables were expressed as a proportion.

Ethical statement

The leadership of the UTH of Point G was informed before starting the study, and study was approved by the ethics committee of the Faculty of Medicine of Dentistry under registration number of n°2019 /76/EC/FMOS.

RESULTS

Global frequency of nosocomial infections

A total of 463 patients were inpatients for at least 48 h in the different departments surveyed and 57 patients (12.3%) had at least one nosocomial infection. We observed 65 episodes of nosocomial infections in the 57 infected patients.

Forty-nine patients had one episode and eight had two episodes, including six patients with two episodes of urinary tract infection and two patients with two episodes of surgical site infection.

Of the different nosocomial infections, 30/65 (46.1%) were urinary infections, 16/65 (24.6%) were surgical site infections, 15/65 (23.1%) were bacteremia, 2/65 (3.1%) were skin infections and 2/65 (3.1%) were pneumonia acquired under mechanical ventilation (PAMV) (Figure 1).

Bacterial strains isolated and resistance pattern

The main microorganisms isolated were Enterobacteriaceae, mainly *E. coli* and *K. pneumoniae*. *E. coli* was the most isolated germ in urinary tract infections, in surgical site infections and in cutaneous infections. *Acinetobacter baumannii* was the most microorganisms isolated in bacteremia, and *Citrobacter freundii* and *Pseudomonas putida* were the germs isolated in PAMV.

Multi-resistant bacteria (MDR) at 63.2% (n=36) were the micro-organisms found in all patients. These were ESBL-secreting *Enterobacteriaceae* (n=21), high-level cephalosporinase (n=13) and methicillin-resistant coagulase-negative Staphylococci (n=2) (Figure 2).

The number of resistant strains corresponds to the summary of the intermediate and antibiotic-resistant strains (Table 1).

DISCUSSION

The sample was exhaustive but there are limits and difficulties such as poorly informed hospitalization records, certain additional examinations were not carried out due to lack of financial means. There could be the possibility of selection bias among patients in the infectious and tropical diseases department where patients were admitted with suspicion of fever. In this study, 54 microorganisms were isolated, Enterobacteriaceae were more frequent (61.1%), followed by non-fermenting Gram-negative bacilli (22.2%) and Gram-positive cocci (11.1%). Our results were consistent with literature data (Pilly, 2015). In our study, the micro-organisms were more isolated in the urine (42.6%), then in parietal suppuration (25.9%) and bacteremia (14.8%), then in skin infections (7.4 %) and

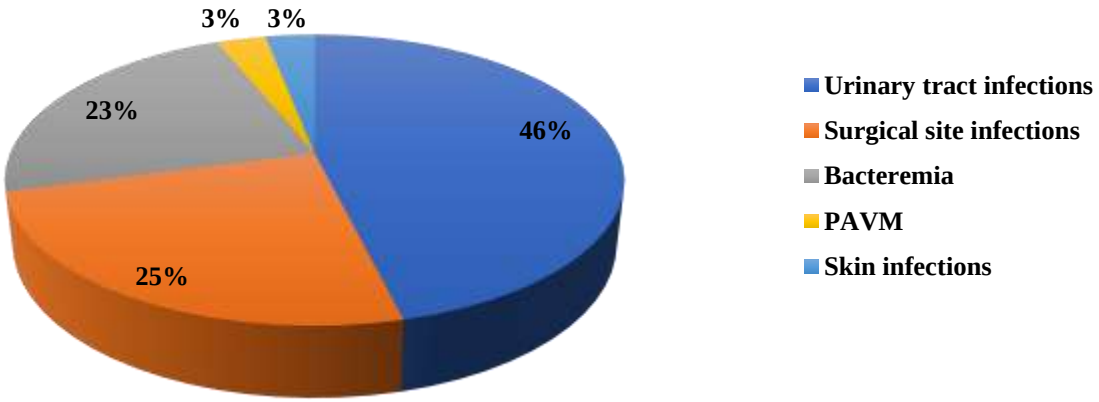

Figure 1. The different nosocomial infections.
PAMV = pneumonia acquired under mechanical ventilation.
Source: Authors

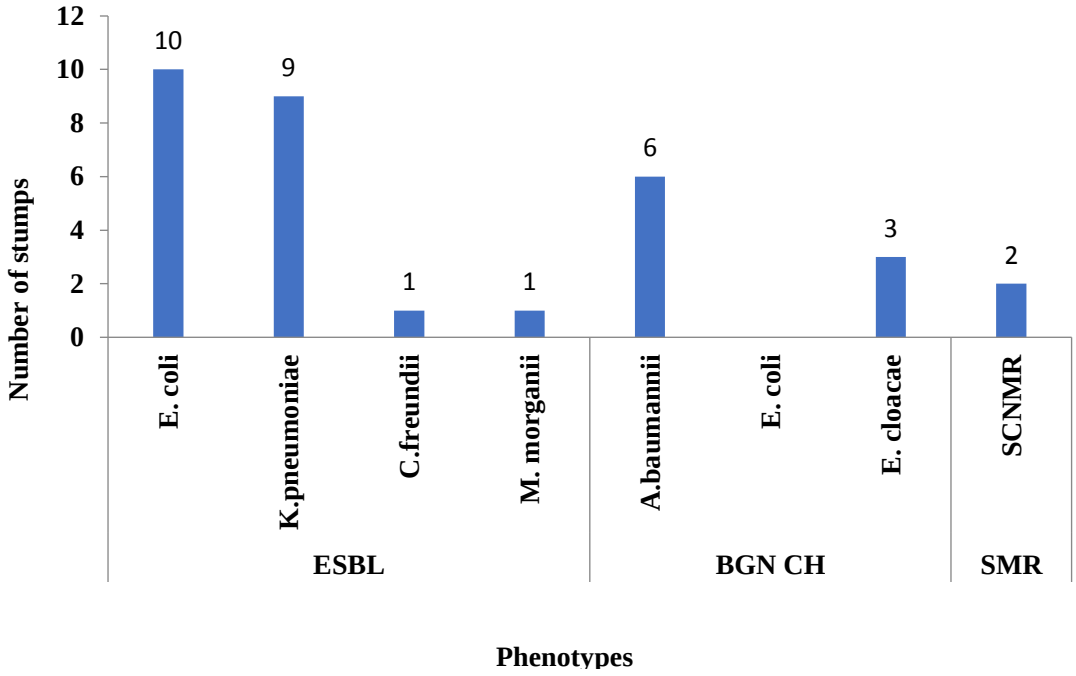

Figure 2. Distribution of multi-resistant bacteria responsible for nosocomial infections. ESBL = extended-spectrum beta-lactamase, BGNCH = Gram-negative bacilli Cephalosporinase hyperproduced, SCNMR= Coagulase Negative Methicillin Resistant *Staphylococcus.*
Source: Authors

PAMV (3.7%). These results confirm those of the national survey on the prevalence of nosocomial infections and healthcare-associated infections in France in 2017 (Daniau et al., 2018), of Issa Maigardie in Mali (Maigardie, 2007) and of Déguénonvo et al. (2015) at the CHU Fann in Dakar in 2015 (Déguénonvo et al., 2015). However, Keita et al. (2016) in Conakry reported rates of urinary tract infections lower than ours (16.1%) (Keita et al., 2016) and they were the second cause of nosocomial infections in their studies. An outline of an epidemic of *E. coli* infection of the surgical site was observed in the general surgery department B in two patients: the two strains of *E. coli* had the same antibiotic type, as well as a sort of cluster of urinary tract infection to *Acinetobacter baumannii* was observed in the urology department in two patients, the two strains had the same antibiotic type. Such clusters can be explained by hand-borne contamination due to poor hand hygiene in these

Table 1. Resistance to antibiotics of the different peces of ESBL.

Antibiotics	Resistance of strains (I+R)		
	***E. co li N*= 16**	***K. pneumonia N*= 10**	***E. cloacae N*= 4**
Beta-lactams			
Amoxicillin	16	10	4
Amoxicillin + aclavulanic acid	16	10	4
Ticarcillin	16	10	4
Cefalotin	16	10	4
Cefotaxim	16	10	4
Ceftazidim	16	10	4
Cefoxitin	14	9	4
Imipenem	1	10	0
Aminosides			
Gentamicin	16	10	4
Amikacin	16	10	4
Quinolones			
Nalidixic acid	16	8	3
Ciprofloxacin	16	9	4
Other antibiotics			
Tetracyclin	13	7	4
Colistin	14	0	4
Chloramphenicol	14	6	3
Sulfonamides	12	3	4
Trimethoprim	11	3	3

Colistin, imipenem and amikacin were the most active antibiotics against *Escherichia coli*, *Klebsiella pneumoniae, Acinetobacter baumannii* and *Enterobacter cloacae*. *Pseudomonas* strains were sensitive to: ceftazidime, imipenem, gentamicin, amikacin and ciprofloxacin. The only strain of *Citrobacter freundii* isolated was resistant to all the molecules tested and *Morganella morganii* was sensitive only to amikacin.
Source: Authors

departments. Such epidemics had not been described at the Point-G University Hospital.

Bacterial resistance to antibiotics has been considered since 2014 by the WHO as a public health priority (Durand et al., 2016). In this study, the frequency of multi-resistant bacteria (MDR) was very high (66.7%) unlike other studies which are between 10.3 to 32.9% in the USA depending on the center, 13% in Europe, and 29.26% in the Maghreb (Harris et al., 2013; Derde et al, 2014; Bourich, 2011). However, in Africa the very often irrational use of antibiotics may explain this high rate. Moreover, in intensive care, as in the majority of long-stay hospitalization units, the prescription of antibiotics is very high, all the studies convergent recognize a decisive role for prior antibiotic therapy as a major factor in the appearance of hospital flora with resistant bacteria: either antibiotic therapy for more than 24 h in the previous days, or less recent but prolonged antibiotic therapy (BMR-Raisin, 2016; Diakity, 2010). Among the MDR, we isolated the broad-spectrum beta-lactamase-secreting Enterobacteriaceae (ESBL), the main species of which were *Escherichia coli* and *Klebsiella pneumoniae*. Bacteria secreting hyper-produced cephalosporinase were *Acinetobacter baumannii*, *Enterobacter cloacae* and *E. coli*. Methicillin-resistant coagulase-negative staphylococci (Figure 2). Maigardie in 2007 in Mali (Maigardie, 2007), Keita et al in Guinea in 2016 (Keita, 2016), Durand A. et al. in France observed the same results as us. Similar results were also observed in other studies in 2016 (Durand et al., 2016). All of our strains of *E. coli, K. pneumoniae* were sensitive to amikacin, (13/16), colistin and (10/16). The sensitivities of *E. coli* for these antibiotics have been constant in Mali since 2010 as shown by the studies of Zitti and Diakité (Diakity, 2010; Ziti, 2014). All of our *E. cloacae* strains were sensitive to colistin and amikacin in this work.

The strains of *Acinetobacter baumannii* found were all multiresistant but were sensitive to colistin and amikacin. This activity was comparable to which found by Diakité with 100% activity for each molecule (Pellegrino et al.,

2002).

Ticarcillin, imipenem, ceftazidim, gentamicin, amikacin and ciprofloxacin had very good activity on all strains of *Pseudomonas* as reported by Diakité (Diakity, 2010) in his work with similar results for all these molecules except for ciprofloxacin where the activity was borderline (66.7%) and ceftazidim (33.3%) for which the activity was less. Zitti (2014) in his study had reported a limited activity of ceftazidim (53.3%) and a lower activity of amikacin (46.7%), gentamicin (40%) and ciprofloxacin (20%) on strains of *P. aeruginosa*. Indeed, *Pseudomonas* is bacteria that combine many mechanisms of resistance to antibiotics, requiring regular analysis of their activity. In addition, they are implicated in several cases of nosocomial infections, particularly in intensive care units (Giuliani et al., 2005).

Conclusion

This study reported that colistin and amikacin still retain very good activity on all strains of Enterobacteriaceae in healthcare settings despite a high level of multi-resistant bacteria. These data show the need for infection control interventions in Mali and a rigorous and effective application of disinfection procedures and better hospital hygiene as well as the rational use of antibiotics. These measures will enable effective infection control and monitoring of these interventions as an indicator of quality of care.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Characterizing sweet potato RDR, AGO and DCL genes and potential involvement in defense against virus infections in sweet potato

Peter Wasswa[1]*, Alexander Ssamula[2], Settumba B. Mukasa[1], John Ssengo[3] and Victor Gaba[4]

[1]School of Agricultural Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda.
[2]Department of Crop Inspection and Certification-Ministry of Agriculture, Animal Industry and Fisheries, P. O. Box 102, Entebbe, Uganda.
[3]Department of Plant Pathology, Entomology and Microbiology, College of Agriculture and Life Sciences, Iowa State University, Ames, IA, United State.
[4]Department of Plant Pathology and Weed Research, Agricultural Research Organization- The Volcani Center, Rishon LeZion 7505101, Israel.

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Characterizing anti-virus genes in sweetpotato is a vital step in mitigating yield loss due to virus infections. This work lays an insight into the structure and expression of key anti-virus genes. Related plant-based anti-virus genes were used as reference to mine key sweetpotato genes from various databases. BLASTN and BLASTP for transcripts was done for evaluation of phylogenetic relationship. Eight genes were identified: RNA dependent RNA polymerases (RDR) 1, 2, 5 and 6; Argonaute 1; and Dicer-like 1, 2 and 4, with more variants for RDR1 transcripts. Phylogenetically, RDR defense genes evolved more recently than other genes. Given the big number of variants and recent evolution of RDRs, further analysis for DLDGD or DFDGD catalytic domains, organization of coding sequences and gene expression were done on RDRs. DLDGD or DFDGD were observed in RDRs with the exception of IbRDR1c_Ch1_1623 and RDR2_Chr2_1059. RDR1 variants revealed varying exon-intron organization, and the IbRDR1c_Ch1_1623 transcript had no introns. High titres for IbRDR1a_Chr8_3068, IbRDR1b_Chr8_3014 and IbRDR1d_Chr8_1149 were observed in SPVC-infected plants suggesting these RDRI variants are involved in resistance against virus infection. The titre of IbRDR1c_Chr1_1623 was not affected. This study offers an opportunity for molecular breeding and selection of cultivars for distribution to farmers.

Key words: Virus defense genes, gene silencing, RNA dependent RNA polymerases (RDR), catalytic domain, titre.

INTRODUCTION

Studies on sweetpotato virus defense genes have received limited attention. This is against the fact that a wide range of viruses attack sweetpotato. For instance, in East Africa, several sweetpotato viral infections have been identified. These include *Sweet potato feathery mottle virus* (SPFMV: *Potyvirus*; Potyviridae), *Sweet*

*Corresponding author. E-mail: wpeter.wasswa@mak.ac.ug. Tel: +256(0)782762081.

potato chlorotic stunt virus (SPCSV: *Crinivirus*; Closteroviridae), Sweet potato leaf curl sweepoviruses (SPLC: *Begomovirus*; geminiviridae), *Sweet potato mild mottle virus* (SPMMV: *Ipomovirus*; Potyviridae), *Sweet potato chlorotic fleck virus* (*Carlavirus*; Flexiviridae) and *Sweet potato caulimo-like virus* (SPCaV: *Caulimovirus*; Caulimoviridae) (Carey et al., 1998; Mukasa et al., 2003; Aritua et al., 2007; Wasswa et al., 2011). East Africa being a tropical environment with high vector populations lays fertile grounds for infections of newly established gardens/farms, therefore potentially undermining the efforts of increased sweetpotato production. These viral infections cause yield loss of up to 50% in single infections (Adikini et al., 2015). The yield loss may increase up to 98% when two or more viruses co-infect, as in the case of Sweetpotato virus disease that is due co-infection of SPFMV and SPCSV (Gibson et al., 1998).

Some East African varieties which have been in the field for decades, have never gone through any anti-viral therapy but are generally virus-free and have yields which have not apparently declined. For instance, in Tanzania, Tairo et al. (2004) reported that 38 of 73 (52%) asymptomatic field plants were sero-negative to viruses. In Kenya, Ateka et al. (2004) showed that 477 of 638 (75%) asymptomatic field plants were virus-free. In Uganda, of the 200 symptomless plants, only 9 (4.5%) were found to be infected with SPFMV and 5 (2.5%) with SPCSV (Aritua et al., 2007). In Rwanda, Njeru et al. (2008) reported that 71 of 103 (69%) asymptomatic field plants were virus-free. This rarity in infection appears to be due to host resistance. Several studies show that the crop tends to 'heal' itself where it fights off the virus, becoming asymptomatic and eventually virus free (Gibson et al., 2014; Gibson and Kreuze, 2015; Ssamula et al., 2019A).

In plants, viral resistance has been envisaged to be due to basal, innate immunity and RNA silencing (RS) or RNA interference (RNAi) (Muhammad et al., 2019). In sweetpotato, Gibson and Krauze (2015) further postulated that viral resistance is due to gene/RNA silencing. Gene or RNA silencing is a virus surveillance system present in all plants that involves small interfering (si)RNAs which are produced through the coordinated function of RDR-DCL-AGO genes (Borges and Martienssen, 2015; Bologna and Voinnet, 2015). The siRNAs join a RISC (RNase Induced Silencing Complex) that bind to cognate sections of foreign RNA such as plant viruses, 'chopping them up' at each binding position to produce yet more siRNAs (Incarbone and Dunoyer, 2013).

Interestingly, plant genome-encoded RDRs have been reported to be involved across several resistance responses (Rakhshandehroo et al., 2017; Leibman et al., 2022). Plants encode six RDR variants, where RDR1 is the most dominant in gene silencing (Donaire et al., 2008; Garcia-Ruiz et al., 2010). RDR1occurs in all investigated plant species and production of small-RNA during RNA virus infection is activated by RDR1 activity (Cao et al., 2014). Apart from virus defense, RDR1 is involved with responses to other pathogen defense, abiotic stress and defense against insect herbivores (Pandey et al., 2008; Matsui et al., 2017; Polydore and Axtell, 2018). RDR1 is also known to regulate microRNA levels and plays a role in regulating important endogenous genes via mRNA-mediated DNA methylation, and is associated with the biotic and abiotic stress response (Polydore and and Axtell, 2018). Understanding the nature/characteristics of viral defense genes, especially RDRs, opens avenues of mitigating the potential sweetpotato production challenges caused by viruses. In this paper, we report on the potential virus resistance genes and amino acid motifs, structural organization and expression of RDR1 upon virus infection in sweetpotato.

MATERIALS AND METHODS

Mining genes associated with virus resistance

Virus resistance gene sequences for model plants *Arabidopsis thaliana* and *Nicotiana benthamiana* were used as reference to conduct the NCBI searches for related plants with similar genes. These reference sequences were used to construct partial sequences for sweetpotato. Partial sequences were obtained using a blind search and query from RNA seq data previously deposited in NCBI (SRA-database). RNA seq data of virus infected sweetpotato was run on a virtual linux platform through command line. The seq data was *de novo* assembled using de Bruijn graph approach (Roumpeka et al., 2017) into contigs while using previously mined sequences as reference. For quality assurance, a scaffold length of 50% was used (N50) according to Mäkinen et al. (2012) procedure using MetaQUAST and MetaVelvet software (Namiki et al., 2012). These helped to detect putative structural variants and misassemblies which were appropriately trimmed. The obtained sequences were re-matched to reference sequences in NCBI alignment tool for validation. The yielded short reads and assemblies (partial sequences) were also mapped and analysed for expression profiles using the Gene Expression Omnibus in NCBI to validate expressions of these genes. Also MetaGene gene finding software (Noguchi et al., 2008) and FragGeneScan (Rho et al., 2010) were used to validate the reference sequences to the genes. The RNA short reads were reverse transcribed to DNA sequences using platform/tool on biomodel.uah.es. The DNA sequences were re-checked by transcribing back to RNA and homology validated using NCBI. A homology level >95% was used. This validated that the reverse transcribed product (that is DNA) was highly identical to RNA and thus could be used for further evaluations.

Partial DNA gene sequences were BLAST searched on the sweetpotato genome sequence and genome sequences of sweetpotato wild relatives available at http://sweetpotato.uga.edu/. This yielded homologous genomic DNA sequence that contained coding and non-coding sequences. Coding sequences were used in downstream work because they are translated to RNA/protein that potentially code for virus resistance. These coding sequences were used as reference sequences for BLAST searching against a database created using FASTA files. The FASTA files were created by downloading and saving different chromosomal genomic DNA sequences (of sweetpotato) derived from FASTQ sequences from sweetpotato genome database (Yang et al., 2017). The FASTA files were used to create a reference database in BIOEDIT (Hall, 1999) (www.qiagenbioinformatics.com/products/clc-main-workbench) platform and CLC workbench validated using Unipro UGENE

(Okonechnikov, 2012). The partial genes were run at stringency of E^{-10} and E^{-6}; these yielded partial hits of increased sequence lengths (of the initial RNA/nucleotide sequences). Generated partial genes sequences from this process were allocated identifier names and numbers, respectively.

Phylogenetic analysis of sweetpotato anti-virus genes and related genes in other plant species

Phylogenetic analysis was done using translated protein sequences of sweetpotato, its relatives and plants where related viral resistance genes have been reported. During the phylogenetic analysis, random plants were selected as roots. The phylogenetic/evolutionary analysis was done using Phylip Neighbour joining tree building method following the model by Jones et al. (1992) and Bootstrapped with 100 replicates in Unipro UGENE software (Okonechnikov, 2012) and validated in the CLC workbench. This process was done to establish if the predicted proteins were associated or similar to those involved in the processes associated with viral gene silencing.

Amino acid-protein sequence properties of RDRs

Given the big number of variants for RDRs (Table 2) and their recent evolution particularly RDR1 (Appendix: Figure 1), further analysis was done on these RDRs. Our decision to further analyse RDRs was also based on the findings by Leibman et al. (2022) who found melon (*Cucumis melo*) to encode variants of RDR1 genes (CmRDR1a, c1 and c2) whose expression levels variously increased upon infection with various geminiviruses and potyviruses. RDR1 was also reported by various researchers as one of the main enzyme of all RDRs involved in RNA silencing (Qi et al., 2009; Garcia-Ruiz et al., 2010).

In order to establish whether an amino acid derived from RDR coding sequences is potentially involved in viral defense, reference was made to Wassenegger and Krczal (2006) and Hua et al. (2021). The presence of C-terminal canonical DLDGD or DFDGD amino acid motifs was analysed for sweetpotato RDR based coding sequences (genes). Here, the nucleotide sequences of different coding sequences were translated to amino acid (protein) sequences using the *ExPASy* (Gasteiger et al., 2003) translation tools and verified using Open Reading Frame Finder on NCBI platform. The amino acid sequences were aligned in Unipro UGENE and verified in CLC workbench and BIOEDIT platforms. This alignment was also against related RDRs from other plants. Further structural amino acid-protein validations were made against sampled RDRs, to compare the relation with other plants. This was done using the Protein Data bank (https://www.rcsb.org/) and ORION (Ghouzam et al., 2016). These processes led to validation for presence of the canonical DLDGD or DFDGD amino acid motifs in sweetpotato RDRs. Further to this, a conserved domain search for the amino acids/proteins was made in NCBI's conserved domain resource (https://www.ncbi.nlm.nih.gov/Structure/cdd). This was aimed at establishing if the RDR gene family possessed those conserved sites that are historically involved in viral resistance.

Predicting the structure/orientation of DNA coding sequence of RDR1

To analyse sweetpotato RDR1 orientation, a BLAST search was done against the sweetpotato relatives *Ipomoea triloba* and *Ipomoea trifida* available at the genome site http://sweetpotato.uga.edu/. Reference sequences used were the coding sequences of some key genes (variants) of RDRI where significant variations had been observed. The BLAST search revealed homologous sequences for the sweetpotato relatives against particular coding sequences of the potential viral resistance genes. A phylogenetic analysis using coding sequences was performed for the said sequences, to obtain the closest relative of sweetpotato. This was done using Unipro UGENE platform and validated using CLC-workbench and NCBI tree construction options. The nucleotide sequences of the closest relative was aligned to the coding sequence of sweetpotato in BIOEDIT and validated in Unipro UGENE platform. This revealed the structural organization of sweetpotato coding sequences of key RDR1 variants. It also revealed variational arrangements/deviations from the closest relative. Validations of different sequence compositions of exons and introns were run in NCBI to cross check their functionalities. Structural annotational representations/predictions of nature of the coding sequences were then constructed.

Quantification of RDR1 in *Sweet potato virus* C-infected cultivar Beauregard plants

Quantification of RDR1 was done at Agricultural Research Organisation (ARO) – The Volcani Center, Israel in 2019. *Sweet potato virus* C (SPVC) potyvirus was used as it was the only potyvirus that could be accessed at ARO. Single SPVC infections were established by side grafting naturally infected Beauregard shoot tips from field to 10 healthy plants of cultivar Beauregard in a screen house. Plants were established from cuttings taken from the inoculated Beauregard plants, one week after inoculation, to avoid any effects from the virus infector cuttings. Composite leaf samples from top (3rd leaf from top), middle (5th leaf from top) and bottom parts (most bottom leaf) of the stem of the 3 successfully inoculated plants were separately collected for each plant at the end of 2 weeks. Three healthy mock-inoculated Beauregard plants were included as control. The collected samples were temporarily stored in a cold chamber with freezers at -80°C until they were needed for RNA extraction.

RNA was extracted from healthy and SPVC-infected plants using the TRI Reagent protocol, following the supplier's manual (Bio Labs, Jerusalem, Israel). Nucleic acids were quantified using a NanoDrop-ND-1000 spectrophotometer (Thermo Scientific; Bargal Analytical Instruments, Airport City, Israel). The samples were standardised to 500 ng μl^{-1} using molecular grade water. Complementary DNA (cDNA) was synthesised using Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, Tamar, Israel), following the manufacturer's manual.

Plants were confirmed for infection (Figure 1) using RT-PCR procedure described by Ssamula et al. (2019A). The remaining cDNA was used for determining RDR1 titre (IbRDR1a_Chr8_3068, IbRDR1b_Chr8_3014, IbRDR1c_Chr1_1623 and IbRDR1d_Chr8_1149) using SYBR green method of RT-qPCR. The 25 µl SYBR green qPCR reaction mixture consisted of 12.5 µl of SYBR, 8.5 µl of molecular grade water, 0.75 µl of 5 mM of each primer (Table 1) and 2.5 µl of cDNA. A negative control (molecular grade water) and a housekeeping gene [cytochrome oxidase (cox)] (Weller et al*.,* 2000), were included on the plate (twin.tec PCR plate 96, skirted) and each sample was duplicated to reduce pipetting errors. Plates were sealed with optical adhesive covers (Applied Biosystems). The reaction was performed on Mastercycler® ep realplex Sequence Detection System and qPCR thermal cycler conditions used include 95°C for 15 min (SYBR activation) followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 54°C for 30 s and extension at 72°C for 30 s. Relative RDR1 titre data were analysed from the raw fluorescence data [Ct values at which a change in normalised reporter (ΔRn) crosses the threshold] using the $2^{(\_Delta\ Delta\ C(T))}$ method (Livak and Schmittgen, 2001). The fold change RDR1 titre (target gene) relative to the reference gene (Cox) was determined by the equation: $Cq = 2^{-\Delta\Delta Ct}$: $Cq = 2^{-}[(Cq_{\text{target gene}}) - (Cq_{\text{reference gene}})] - [(\text{mean } Cq_{\text{target gene}}) - (\text{mean } Cq_{\text{reference}}$

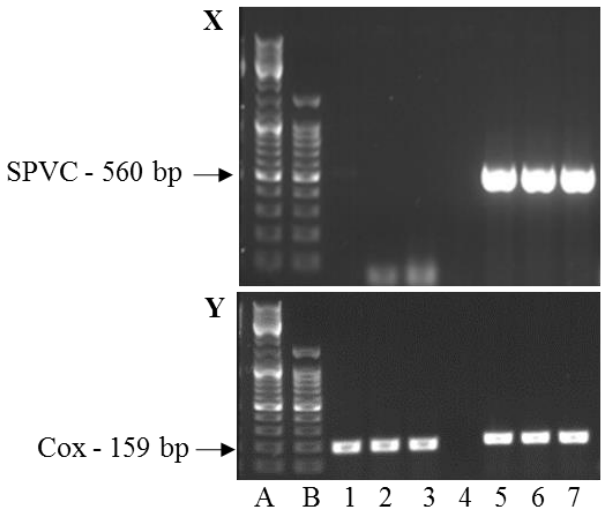

Figure 1. Gel picture showing SPVC amplified products in sweetpotato cultivar Beauregard plants. Plate X depicts SPVC gel picture and plate Y depicts gel picture of the host *Cytochrome C oxidase* reference gene. Lane A = 1kb ladder, B = 100bp ladder (frequently used), 1-3 = healthy plants of cultivar Beauregard, 4 = negative (water) control, and 5-7 SPVC-infected plants of cultivar Beauregard.
Source: Authors

Table 1. Primer sequences used in the study.

Primer name	Sequence (5'-3')	Source
IbRDR1a_Chr8_3068-F	CATCGAGACTTATAGCAGCCG	This study
IbRDR1a_Chr8_3068-R	GGATAATGGCGCAACACACACGATTC	
IbRDR1b_Chr8_3014-F	CTACAAGAAAGCAGAAGGCTCCA	This study
IbRDR1b_Chr8_3014-R	TATCCAGACTAATAGCAGCAGA	
IbRDR1c_Chr1_1623-F	ATCTCATCTGCCTGTAAATA	This study
IbRDR1c_Chr1_1623-R	TTTGATAGAACACCGTACTT	
IbRDR1d_Chr8_1149-F	GTTGGGACCCTGACCTTATT	This study
IbRDR1d_Chr8_1149-R	CTGGAAGCGATTTGGATG	
Cox F	ACTGGAACAGCCAGAGGAGA	Park et al. (2012)
Cox R	ATGCAATCTTCCATGGGTTC	

Source: Authors

$_{gene}$)], where Cq = cycle quantity, ΔΔCq = differences in Cq values between the target gene and reference gene.

RESULTS

Genes associated with virus resistance

According to the gene prediction process, eight genes were obtained. These included four RDRs (RDR 1, 2, 5 and 6); one Agonaute 1 (AGO1); and three Dicer-like (DCL) genes 1, 2 and 4 (Table 2). These sweetpotato genes had variants; RDR1 had four variants, followed by DCL2 with three variants, and RDR5 and DCL1 with two variants each. Other genes had one form (that is, no variant). All the three gene families of sweetpotato had fewer variants than sweetpotato relatives *I. trifida*,

Table 2. Sweetpotato (*Ipomoea batatas*) predicted genes, variants, chromosomal locations and their similarity to *Ipomoea* relatives.

Detected gene	Evaluation on the genome (Yang et al., 2017)			Proposed variant identity	Variants/alternative splice forms/isoforms in *Ipomoea* spp. relatives based on HITS, E-Value and identity			Cumulative no. of variants
	No. of variants	Chromosomal locations	Potential size (nt)		*I. trifida*	*I. triloba*	*I. nil*	
IbRDR1	4	Chr 8	3068	IbRDR1a_Chr8_3068	1	2	1	4
		Chr 8	3014	IbRDR1b_Chr8_3014	1	2	1	4
		Chr 1	1623	IbRDR1c_Chr1_1623	2	4	3	9
		Chr 1	1149	IbRDR1d_Chr8_1149	1	2	1	4
IbRDR2	1	Chr 3	1059	IbRDR2_Chr3_1059	2	2	1	5
IbRDR5	2	Chr 14	2671	IbRDR5a_Chr14_2671	2	5	1	8
		Chr 11	707	IbRDR5b_Chr11_707	2	3	1	6
IbRDR6	1	Chr 10	786	IbRDR6_Chr10_786	1	1	2	4
IbAGO1	1	Chr 3	2201	IbAGO_Chr3_2201	1	1	1	3
IbDCL1	2	Chr 1	3661	IbDCL1a_Chr1_3661	2	1	1	4
		Chr 9	3808	IbDCL1b_Chr9_3808	3	2	1	6
IbDCL2	3	Chr 12	2059	IbDCL2a_Chr12_2059	1	2	4	8
		Chr 13	1685	IbDCL2b_Chr13_1685	5	4	1	10
		Chr 6	1152	IbDCL2c_Chr6_1152	5	5	1	11
IbDCL4	1	Chr 8	2775	IbDCL4_Chr8_2775	1	1	1	3

Ib = *Ipomoea batatas*; RDR = RNA dependent RNA polymerase; AGO = Agonaute; DCL = Dicer-Like; nt = nucleotides. Potential variants/number of variants were obtained by blasting to MSU site for *I. triloba* and *I. trifida*. Considerations were made based on Top Query coverage greater than 80%, Expected value of 0.0 and Identity greater than 80%. For *I. nil* variants with hits greater than 85% identity were considered using NCBI BlastN.
Source: Authors

Ipomoea nil and *I. triloba* (Table 2). In sweetpotato, chromosome 1 and 8 had the highest number of defense genes while other chromosomes had two or one gene. All predicted sweetpotato defense genes had resemblances with genes of wild relatives (Table 2).

Phylogenetic relationship of anti-virus genes in sweetpotato and other plant species

Generally, the virus defense genes of sweetpotato closely related to those of *Ipomoea* species wild relatives, while distantly related to those of other plants. The RDR1 of *Ipomoea* relatives evolved much earlier than those of *Ipomoea batatas*. Of the sweetpotato RDRs, IbRDR1c_Chr1_1623 evolved earlier than the rest. The RDR1 proteins of *Ipomoea* spp. clustered in the same clade. Interestingly IbRDR1d_Chr1_1149 has recently evolved and sequence related to that of Citrus_sinensis_RDR1. (Appendix: Figure 1). The RDR2 protein of most wood-like plants evolved earlier than those of herbaceous plants, of which sweetpotato is part. The *I. batatas* RDR2 protein showed evolutionary lineage from *I. triloba*; with close relationship with *I. trifida* RDR2 protein (Appendix: Figure 2). The RDR5 proteins of *Ipomoea* spp. varied within the phylogram. This is shown by an early evolution of one of the *I. batatas* RDR5 species (IbRDR5a_Chr14_2671) although with a distant relation with that of wild relative *I. trifida* (the isoform). Surprisingly, sweetpotato IbRDR5b_Chr11_707 evolved slightly before the RDR5 of its relatives like *I. triloba* and *I. nil*; although not significant from protein of

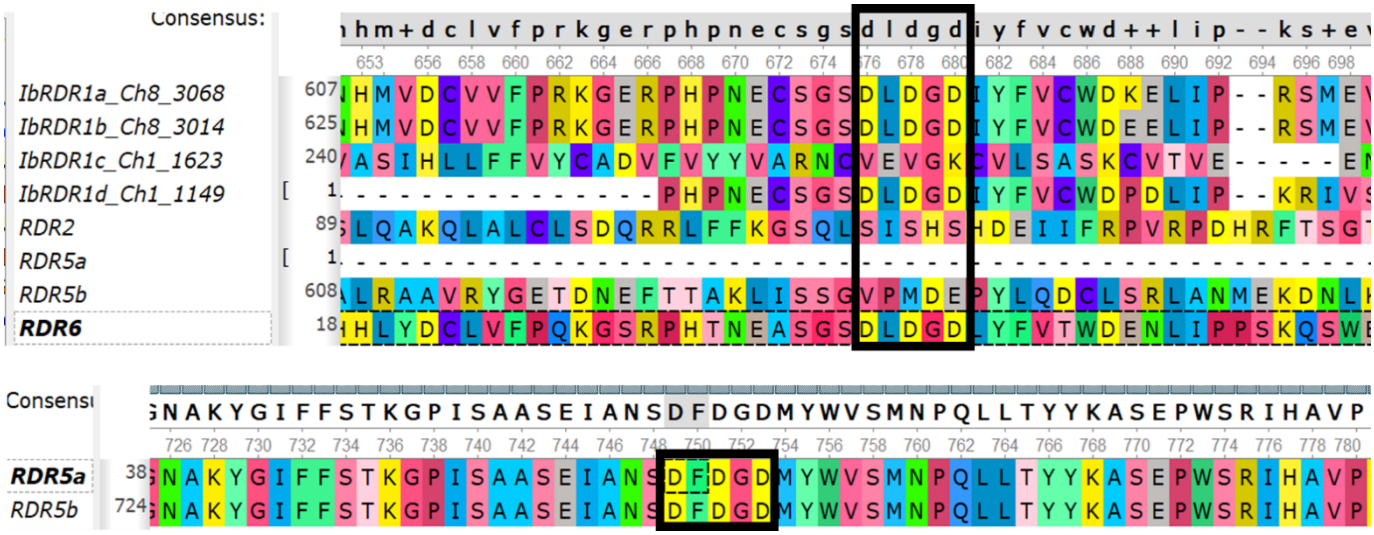

Figure 2. Catalytic domains (in black rectangle) in different sweetpotato amino acids translated from mined defense-gene nucleotide sequences.
Source: Authors

tomatoes (*Solanum lycopersicm*). Other crop RDR5 proteins evolved variously (Appendix: Figure 3). The phylogram showed that sweetpotato RDR6 (IbRDR6_Chr10_786_Protein) evolved earlier than proteins of the relatives *I. nil*, *I. triloba* and *I. trifida*. Of the three sweetpotato relatives, RDR6 of *I. nil* evolved earlier than that of *I. triloba* and *I. trifida*. All these proteins were distantly related to those of other crop plants (Appendix: Figure 4).

AGO 1 protein of sweetpotato (IbAGO 1_Chr3_2201) recently evolved and closely related to *I. trifida* AGO 1 protein, while distantly related to *I. nil* AGO 1. The phylogram showed that sweetpotato AGO1 protein is distantly related to similar proteins from other crop plants like *Nicotiana tabacum*, *Capsicum* species and *Solanum tuberosum* (Appendix: Figure 5). The DCL1 proteins of sweetpotato evolved variously (Appendix: Figure 6). According to the phylogram, IbDCL1a_Chr1_3661 evolved earlier than similar proteins from different crops. However, it was closely related to DCL proteins of tree crops like *Theobroma cacao* and *Vitis vinifera*. Sweetpotato protein IbDCL1b_Chr9_3808 was closely related to DCL1 protein of *I. trifida* and evolved earlier than similar proteins of crops like *Manihot esculenta* and *Jatropha curcas* (Appendix: Figure 6). Sweetpotato IbDCL2c_Chr6_1152 and IbDCL2b_Chr13_1685 evolved earlier than similar proteins of *Ipomoea* relatives (Appendix: Figure 7). IbDCL2a_Chr12_2059 has recently evolved and is related to similar protein of *I. trifida* where they form same clade (Appendix: Figure 7). The IbDCL4_Chr8_2775- protein evolved earlier than DCL4 proteins of different crops like *Coffea eugenioides* and sweetpotato relatives *I. nil*, *I. trifida* and *I. triloba* (Appendix: Figure 8).

Amino acid-protein sequence properties of RDRs

Amino acid catalytic domains/motifs

Catalytic domains of the form DLDGD or DFDGD were observed variously in the RDRs with the exception of IbRDR1c_Ch1_1623 and RDR2_Chr2_1059. The catalytic domain DLDGD was found within IbRDR1a_Ch8_3068, IbRDR1b_Ch8_3014, IbRDR1d_Ch1_1149 and RDR6. On the other hand, catalytic domain DFDGD was found in RDR5a_Chr 14_2671 and RDR5b_Chr11_707 (Figure 2).

Conserved domain evaluation

A conserved domain evaluation for RDRs revealed that all RDRs possessed conserved domains with the exception of IbRDR1c_Ch1_1623. The largest domain was observed in IbRDR1a_Ch8_3068 (of about 572 sequence length, A - coloured blue) and the smallest was in IbRDR2_Chr3_1059 (70 sequence length, D - coloured red) (Figure 3). The domains contained RNA dependent RNA polymerase (RdRP) superfamily for IbRDR1a_Ch8_3068 (A), IbRDR1b_Ch8_3014 (B) and IbRDR5b_Chr11_707 (F); RdRP for IbRDR1d_Ch1_1149 (C), IbRDR5a_Chr14_2671 (E) and IbRDR6_Chr10_786 (G); and RNA-recognition motif (RRM) superfamily for IbRDR2_Chr3_1059 (D) (Figure 3).

Predicted orientation of DNA coding sequence of RDR1

The RDR1 gene family in *I. batatas* was homologous to

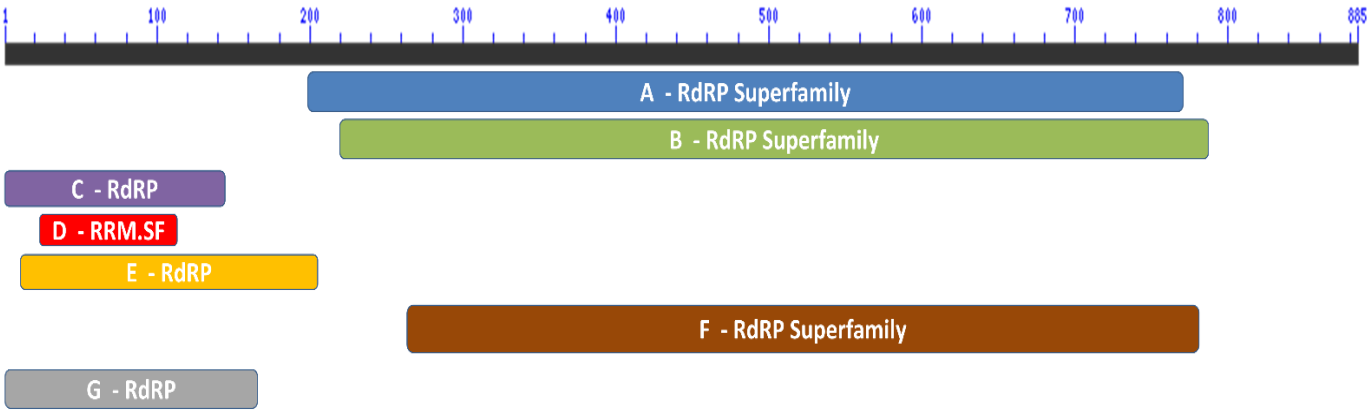

Figure 3. An NCBI architectural coverage of RDR Domains on scale of representative query sequence (for different RDRs). A is RNA dependent RNA Polymerase (RdRP) superfamily domain for IbRDR1a_Ch8_3068; B is RdRP superfamily domain for IbRDR1b_Ch8_3014; C is a RdRP domain for IbRDR1d_Ch1_1149; D is an RNA recognition motif superfamily domain for IbRDR2_Chr3_1059; E is a RdRP domain for IbRDR5a_Chr14_2671; F is a RdRP superfamily domain for IbRDR5b_Chr11_707 and G is a RdRP domain for IbRDR6_Chr10_786.
Source: Authors

those of wild relatives. Analysis of the coding sequences of the RDR1 gene variants revealed differences in orgarnisation of the exons (Figure 4). Four exons were observed in gene transcripts of IbRDR1a_Chr8_3068, IbRDR1b_Chr8_3014 and IbRDR1d_Chr8_1149. IbRDR1c_Chr1_1623 transcript had five exons (Figure 4). The lines between exons showed non-coding regions (introns) (Figure 4); interestingly, the IbRDR1c_Chr1_1623 transcript had no introns. The largest nucleotide sequences were found in IbRDR1a_Chr8_3068 transcript and the least in IbRDR1d_Chr8_1149. A Blast multiple alignment on the different exons revealed that whereas different nucleotide sequences were located within different genes, they were homologous at a level above 95%. This was indicated with similar colouration of exon sequences. Those with different colours were non-homologous (Figure 4).

RDR1 titre in *Sweet potato virus* C-infected cultivar Beauregard plants

In healthy sweetpotato plants, RDR1a, RDR1b, RDR1c and RDR1d were all detectable. RDR1 titre was higher for IbRDR1a_Chr8_3068, IbRDR1b_Chr8_3014 and IbRDR1d_Chr8_1149 in SPVC-infected Beauregard plants than in healthy control plants (Table 3). Highest RDR1 titre was recorded for IbRDR1b_Chr8_3014 followed by IbRDR1a_Chr8_3068 and lowest for IbRDR1d_Chr8_1149. IbRDR1c_Chr1_1623 titre did not vary between SPVC-infected and healthy control plants.

In SPVC-infected plants, IbRDR1b_Chr8_3014, IbRDR1a_Chr8_3068 and IbRDR1d_Chr8_1149 titres were 24.2, 15.7 and 3.3 folds, respectively, compared to healthy control plants (Table 3).

DISCUSSION

Here, we report on sweetpotato genes associated with virus resistance, their phylogenetic relationship, genomic characterization and expression in virus infected plants. This is the first report of RDR genome organization in sweetpotato and RDR expression in healthy and virus-infected sweetpotato plants.

Three gene families, that is, RDR, DCL and AGO were predicted in sweetpotato. These same genes are known to occur in other plant systems where they involve in resistance against various stresses in crop plants (Qin et al., 2018; Cui et al., 2020; Ahmed et al., 2021). Indeed, the RDR, DCL and AGO genes of sweetpotato had resemblance to the defense genes of wild relatives *I. trifida*, *I. nil* and *I. triroba*, and other plants suggesting their involvement in virus resistance in sweetpotato. This resemblance suggests homology during evolutionary development. Coordinated function of RDR-DCL-AGO genes in plants is crucial for processing different classes of small RNAs, which indirectly makes them involved in regulation of diverse biological pathways (Borges and Martienssen, 2015; Bologna and Voinnet, 2015). Members of these three gene families are involved in biogenesis of sRNAs and effective silencing of their targets, viruses inclusive (Bologna and Voinnet, 2015).

Four RDRs 1, 2, 5 and 6; one AGO 1; and three DCLs 1, 2 and 4 resistance genes were predicted, although these three gene families of a hexaploid sweetpotato had fewer variants than in the diploid *I. trifida*, *I. nil* and *I. triroba*. This observation is possible because an organism's genome size does not depend on the number of genes (or chromosomes) it contains (Hou and Lin, 2009). It is, therefore, possible for a more complicated or advanced organism to have less gene variants.

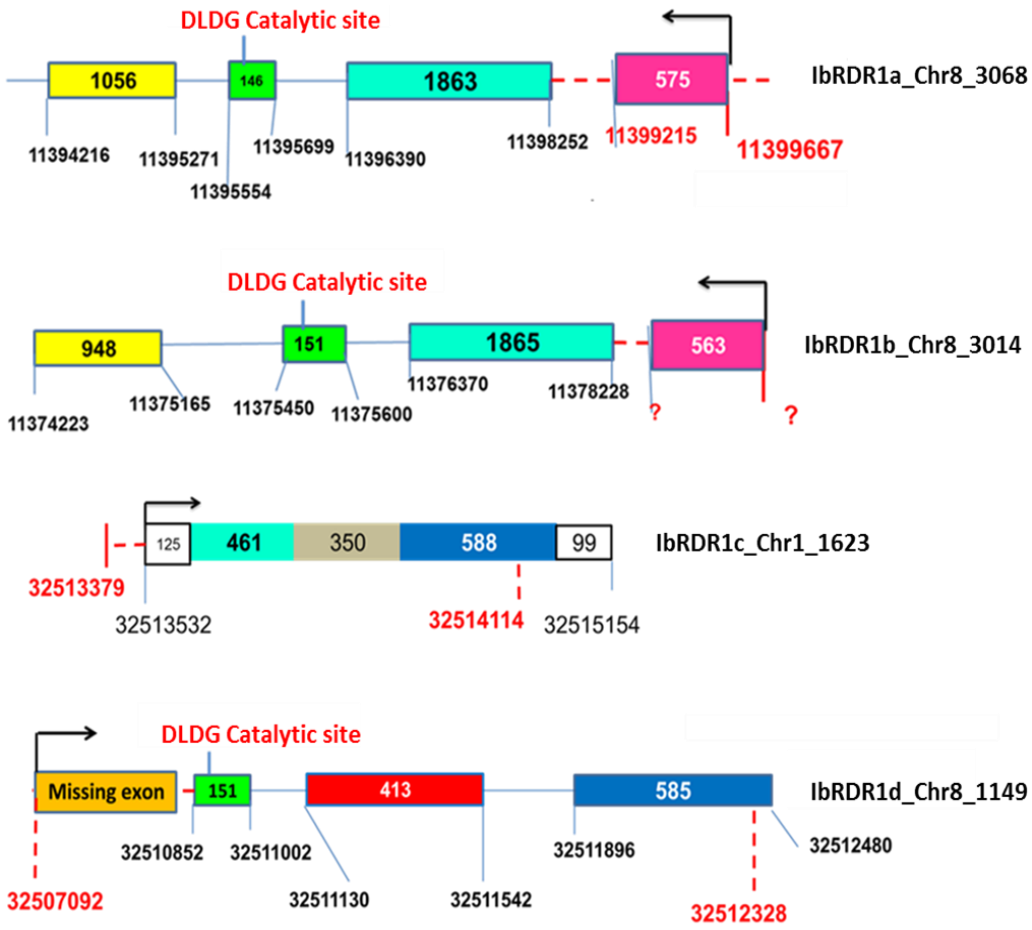

Figure 4. Genome maps of IbRDR1a_Chr8_3068, IbRDR1b_Chr8_3014, IbRDR1c_Chr1_1623 and IbRDR1d_Chr8_1149 genes in sweetpotato. Boxes represent exons and lines indicate introns. The dashed lines represent unknown sequences. Genome maps were based on the relevant databases (http://sweetpotato.uga.edu/ (accessed on 23rd November 2022)). The numbers indicate exon size (nucleotides) and the start codons (ATG) are represented by an arrow. The two numbers at the start and end of the exons indicate start and end points of that exon with reference to sweetpotato chromosomal sequences. For RDR1b and missing exon region in RDR1d sweetpotato chromosomal areas were not yet sequenced hence *I. trifida* sequence was used as reference.
Source: Authors

Table 3. Mean fold change in RDR1 titre (average $2^{-\Delta\Delta Ct}$) at 2 weeks after graft inoculation of cv Beauregard plants with SPVC.

Plant No.	Average $2^{-\Delta\Delta Ct}$			
	IbRDR1a_Chr8_3068	IbRDR1b_Chr8_3014	IbRDR1c_Chr1_1623	IbRDR1d_Chr8_1149
1	23.473	18.572	0.023	2.357
2	27.522	15.927	0.011	4.227
3	21.516	12.706	0.041	3.188
Mean of means	24.2	15.7	0.03	3.3

Source: Authors

Sweetpotato is an autohexaploid species with 90 chromosomes (2n = 6x = 90) and a basic chromosome number of 15 while sweetpotato progenitors *I. trifida*, *I. nil* and *I. triloba* are diploid and with 15 pairs of chromosomes (2*n* = 2*x* = 30) (Isobe et al., 2019). The RDR1 had the highest number of variants. This

observation is in agreement with earlier findings by Ssamula et al. (2019B). The high RDR1 variability probably signifies the dominant role RDR1 plays during viral gene silencing in sweetpotato. Previous research has actually shown that there are six different RDRs; however, RDR1 and RDR6 are the main enzymes that amplify single-stranded RNA from viruses into aberrant dsRNA, which are digested by the host-encoded Dicer-like (DCL) DCL-4 and DCL-2 proteins into 21–22 nts virus-siRNA duplexes (Qi et al., 2009; Garcia-Ruiz et al., 2010).

Each of the chromosomes could have several genes, as was observed in this study, where most defense genes mapped on chromosome 1 and 8. Whereas the largest chromosome of an organism is generally referred to as chromosome 1, the next largest as chromosome 2, and so on, different chromosomes contain different specific genes whereby, each chromosome contains a specific chunk of the genome (Hou and Lin, 2009). Therefore, the variability in gene locations on different chromosomes is not surprising.

The close phylogenetic relationship of the sweetpotato defense genes to the wild relatives is in agreement with findings by Ssamula et al. (2019B), and suggests that these genes acquired their resistance role before separation of the ancestral plants into different taxa. The sustained presence of the defense genes in different plants after divergence into different taxa during evolutionary development further signifies the importance of these genes in plant life of sweetpotato.

The recent evolution of RDR1 protein of *Ipomoea* spp. further points to the significant role RDR1 may be playing in sweetpotato resistance against virus infections. Several earlier studies in other plants have reported an additional role of RDR1 in virus defense (Qi et al., 2009; Garcia-Ruiz et al., 2010). IbRDR1c_Chr1_1623 evolved earlier than the rest of IbRDR1 gene family variants probably indicating this gene variant is less important in virus resistance. Interestingly IbRDR1d_Chr1_1149 has recently evolved and sequence related to that of Citrus_sinensis_RDR1. This is a case of parallel evolution resulting into phenotypic convergence (Nedelcu, 2019). Like sweetpotato, citrus is affected by several viruses some of which are in the same genus e.g, closterovirus (Umer et al., 2019). These viruses, although in different plant species, may require similar defense genes and mechanisms.

The AGO 1 protein of sweetpotato (IbAGO 1_Chr3_2201) recently evolved, yet only IbDCL2a_Chr12_2059 of the DCL variants showed recent evolution. The DCL main role is to digest the aberrant dsRNA from a replicating virus or secondary structure of the virus into 21–22 nts virus-siRNA duplexes (Qi et al., 2009). On the other hand, AGO proteins are catalytic subunits of the silencing complexes that are loaded with small RNAs to execute the sequence specific RNA cleavage by DCLs (Qi et al., 2009; Garcia-Ruiz et al., 2010).

The IbRDR1c_Chr1_1623 variant which was observed to have evolved earlier than the other IbRDR1s did not have catalytic domains of the form DLDGD or DFDGD. This same gene variant did not possess any conserved domain historically relevant to virus resistance. This further suggests IbRDR1c_Chr1_1623 to be of minor or no role in virus defense. The IbRDR2_Chr3_1059 gene variant which also lacked the catalytic domains (DLDGD or DFDGD) possessed a conserved domain but this was only 70 nucleotides long implying that IbRDR2_Chr3_1059 is also of less importance in virus resistance in sweetpotato. The catalytic and conserved domains have been reported to play a positive role in virus resistance functionality (Verlaan et al., 2013). We therefore propose that IbRDR1c_Chr1_1623 and IbRDR2_Chr3_1059 were probably mutated in an ancestor and this probably made sweetpotato susceptible to virus infections. If this hypothesis is correct, these genes could be repaired through techniques like CRISPR/Cas9 and thus improve virus resistance in sweetpotato. However, it could also be true that the other RDRs evolved to enable sweetpotato acquire a certain degree of virus resistance we see today.

The four transcripts of the RDR1 gene family (IbRDR1a, IbRDR1b, IbRDR1c and IbRDR1d) were arranged variously. IbRDR1a, IbRDR1b and IbRDR1d had similar genome organization (intron/exons) with four exons each. This suggests the relatedness in the role these gene transcripts play in virus defense in sweetpotato. The IbRDR1c lacked introns and yet had five exons. As was observed earlier, the IbRDR1c transcript also evolved earlier than the rest of RDRs, and lacked the catalytic and conserved domains. Whereas introns are not expressed, they are important in gene regulation (Shaul, 2017). This, therefore, further confirms that IbRDR1c transcript is generally not functional, despite it having more exons than the IbRDR1a, IbRDR1b and IbRDR1d transcripts.

The RDR1s expressed variously in infected plants. IbRDR1a_Chr8_3068 expressed 24-fold, IbRDR1b_Chr8_3014 increased 16-fold while IbRDR1d_Chr8_1149 was the least increased by 3-fold. IbRDR1c_Chr1_1623 gene expression did not increase upon infection. This confirmed that RDR1c transcript is not functional in sweetpotato virus defense. Observations of increase in expression of RDR1 gene were also made in other crops by previous researchers. For instance, increased expression of RDR1a gene (CmRDR1a) was observed in cucumber upon infection with *Cucumber mosaic virus* where the gene titre increased by 21-fold (Leibman et al., 2022). Interestingly, unlike in our study, in the study by Leibman et al. (2022) CmRDR1a gene titre did not increase in cucumber upon infection with potyviruses *Zucchini yellow mosaic virus* and *Cucumber vein yellowing virus*. This shows differences in the interaction of plants and viruses in different plant species

which subsequently require varying deployment of resistance mechanisms (Yadav and Chhibbar, 2018).

Conclusion

Evaluation of potential genes associated with virus resistance in a commercially important sweetpotato crop certainly helps to contribute to an increase in crop productivity and quality. In the present study, we identified 4 RDRs, 1 AGO and 3 DCLs in sweetpotato genome. Phylogenetic and structural analyses of these gene sequences show differences in arrangement of exons and introns, based on which they can be grouped into distinct clades. Presence of the catalytic domains (DLDGD and DFDGD) indicate the involvement of these genes in virus resistance, and indeed the titre of RDR1a, RDR1b and RDR1d transcripts increased upon infection with SPVC. The genes identified in this study can be used as potential targets for crop improvement for developing virus resistant sweetpotato cultivars.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

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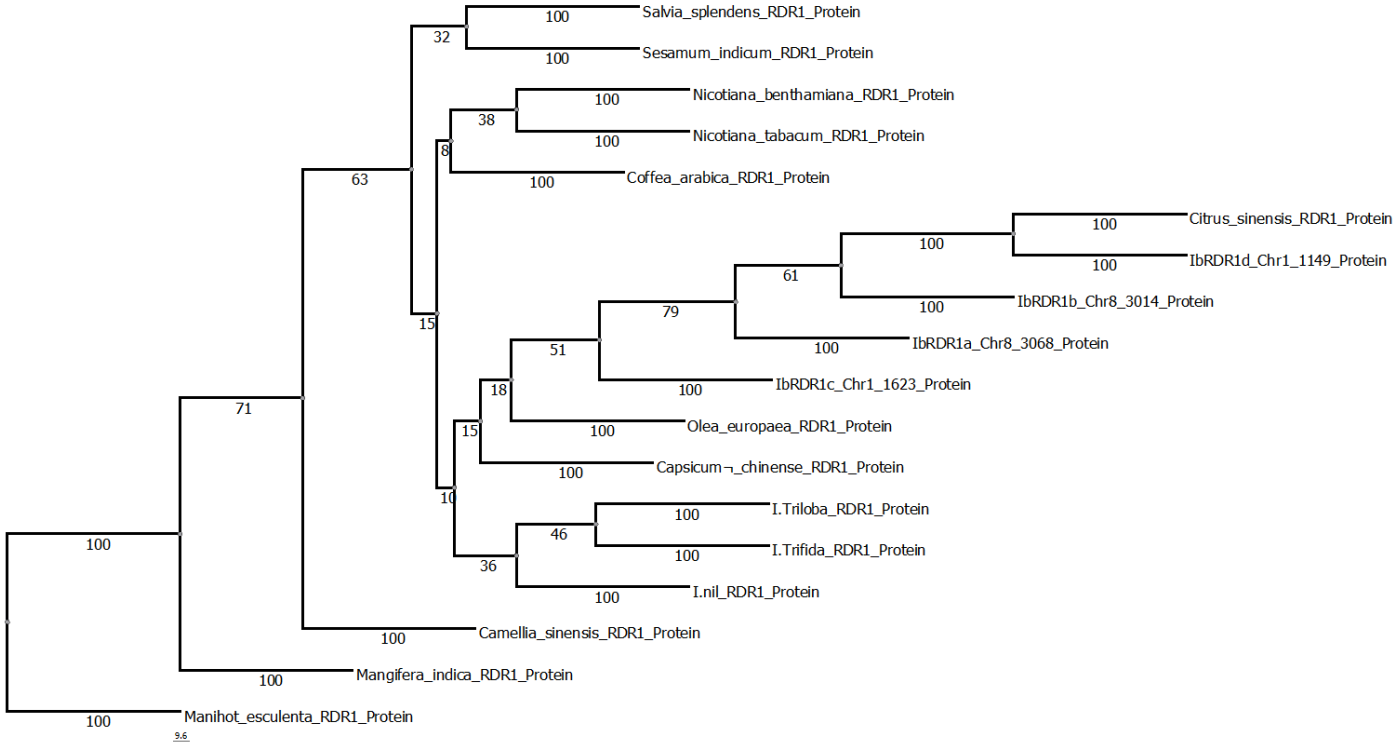
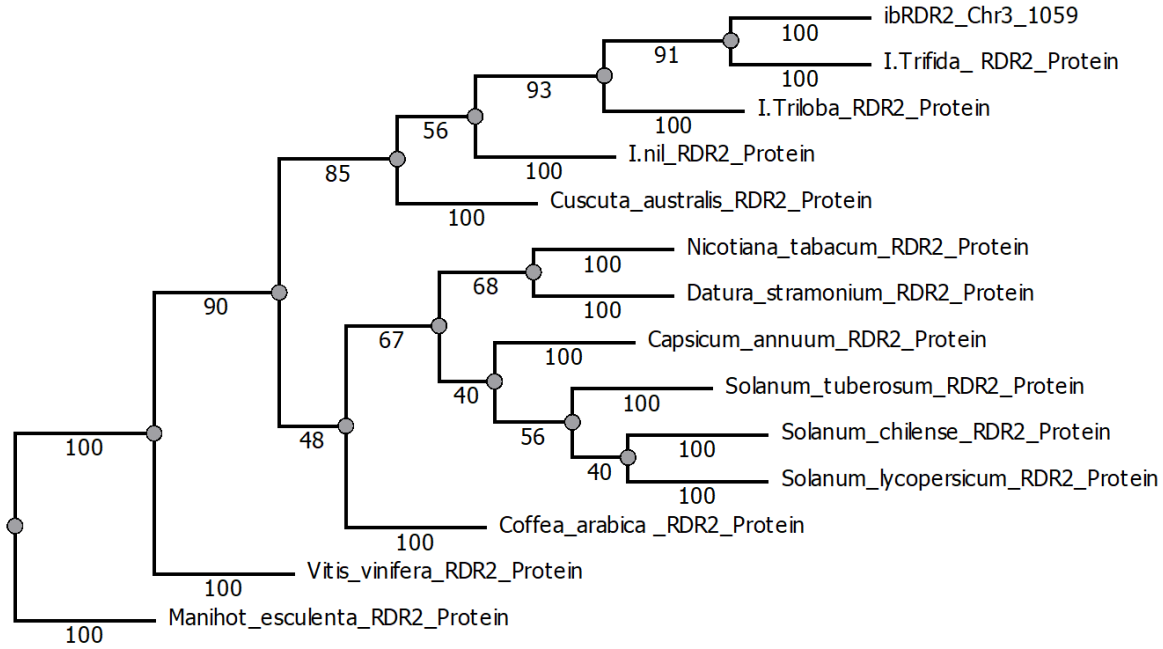
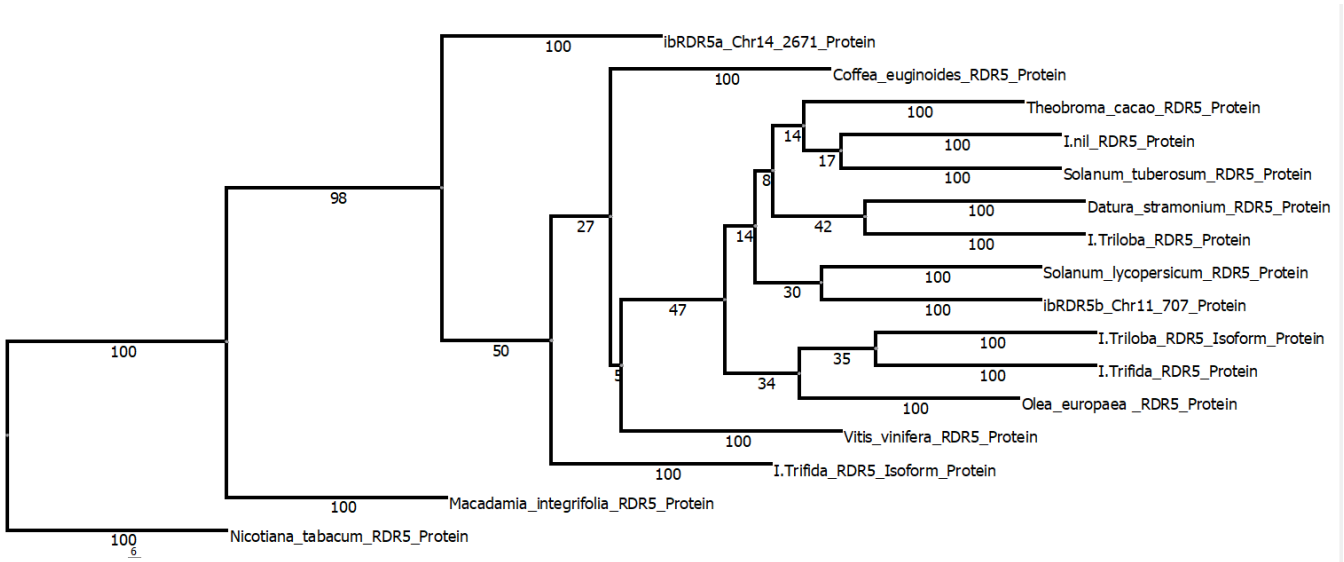
APPENDIX



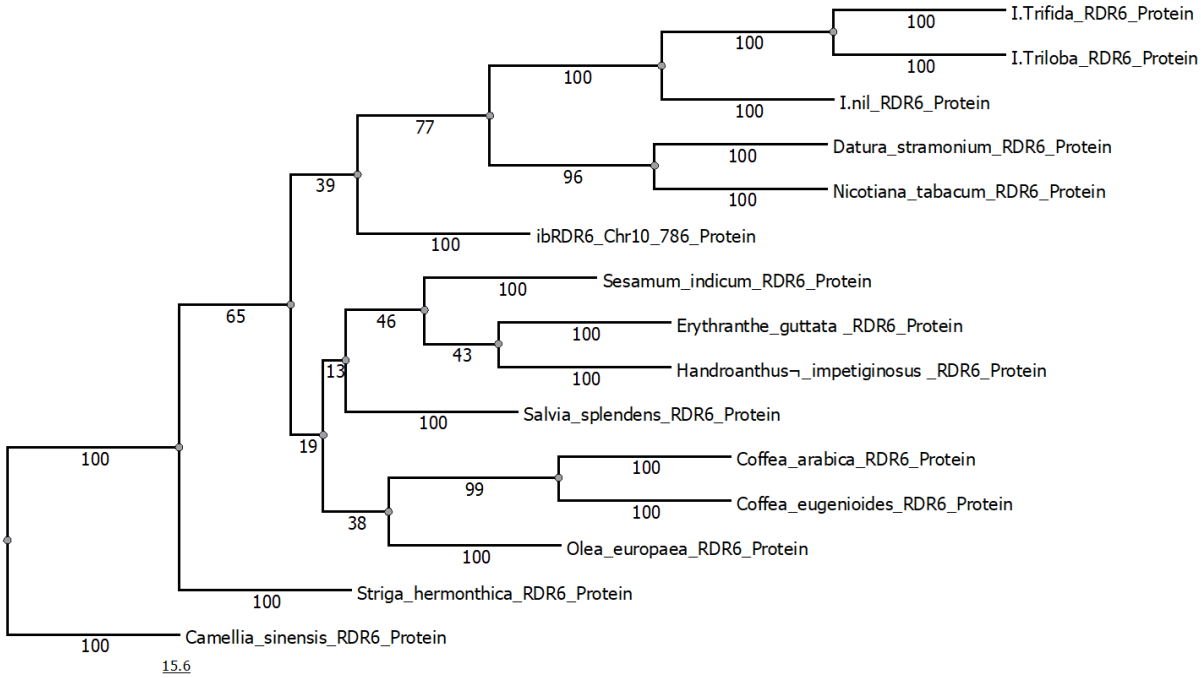
Appendix Figure 1. Phylogenetic tree showing evolutionary relationship of proteins of *Ipomoea batatas* RDR1 and RDR1 from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.



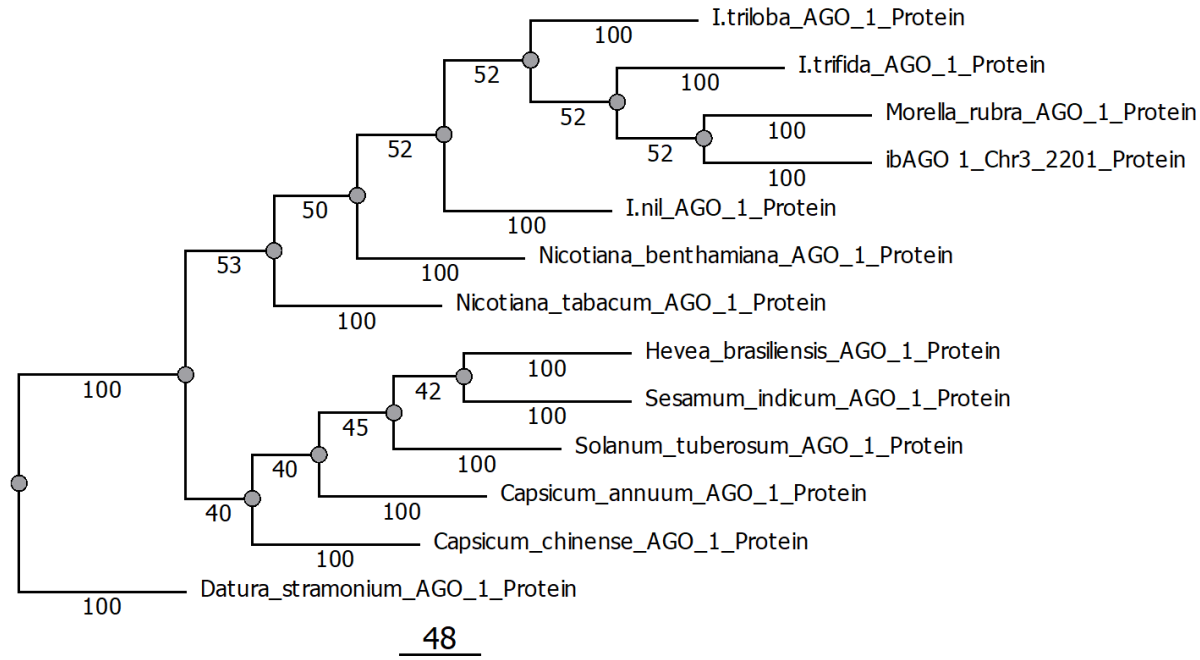
Appendix Figure 2. Phylogenetic tree showing evolutionary relationship of proteins of *Ipomoea batatas* RDR2 and RDR2 from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.



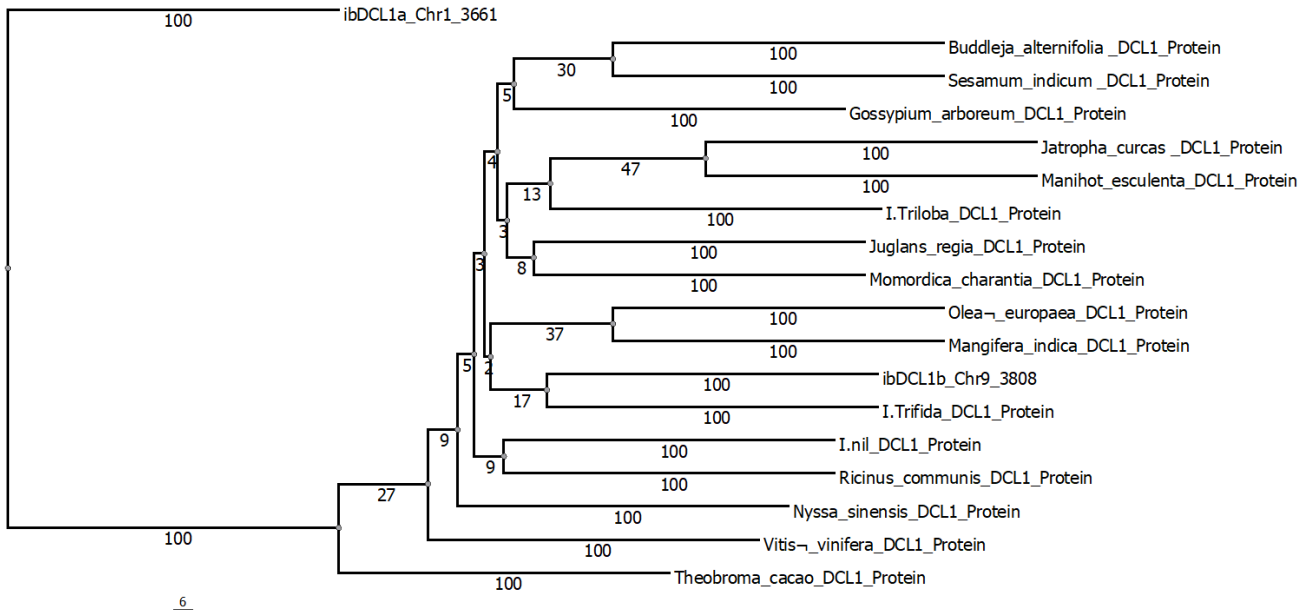
Appendix Figure 3. Phylogenetic tree showing evolutionary relationship of proteins of *Ipomoea batatas* RDR5 and RDR5 from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.



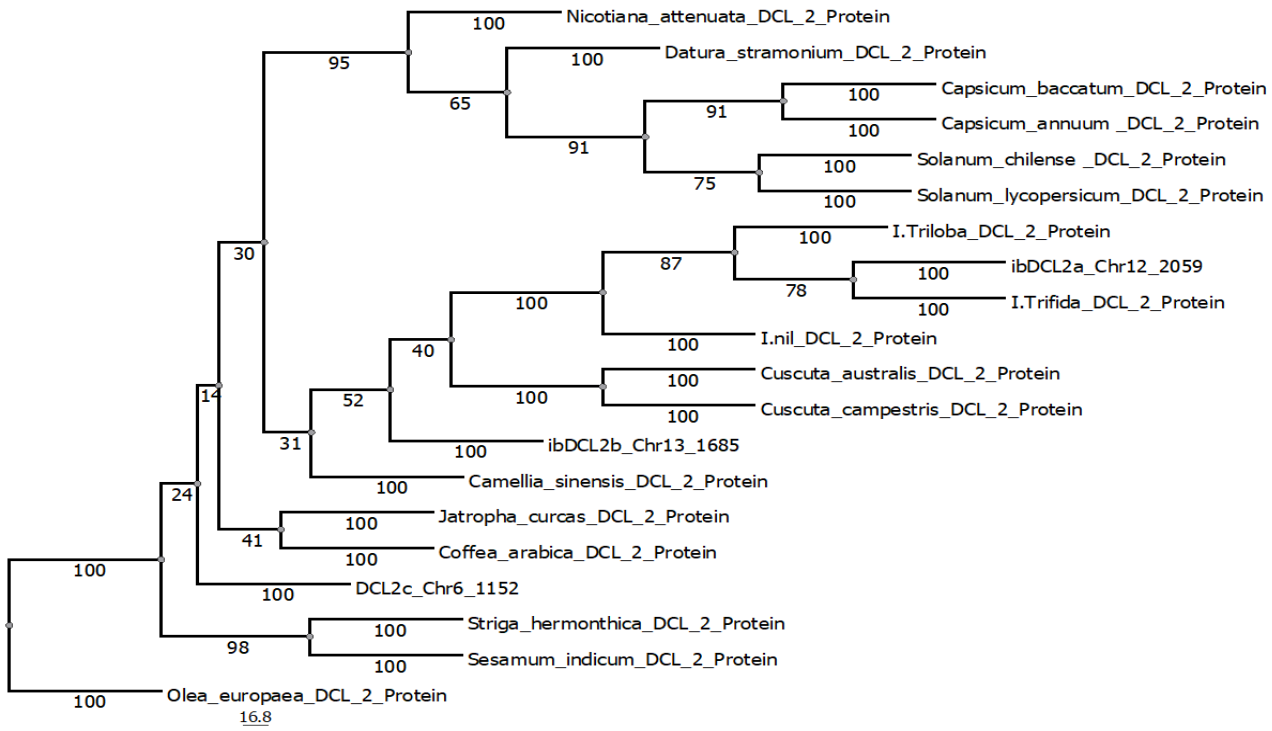
Appendix Figure 4. Phylogenetic tree showing evolutionary relationship of proteins of *Ipomoea batatas* RDR6 and RDR6 from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.



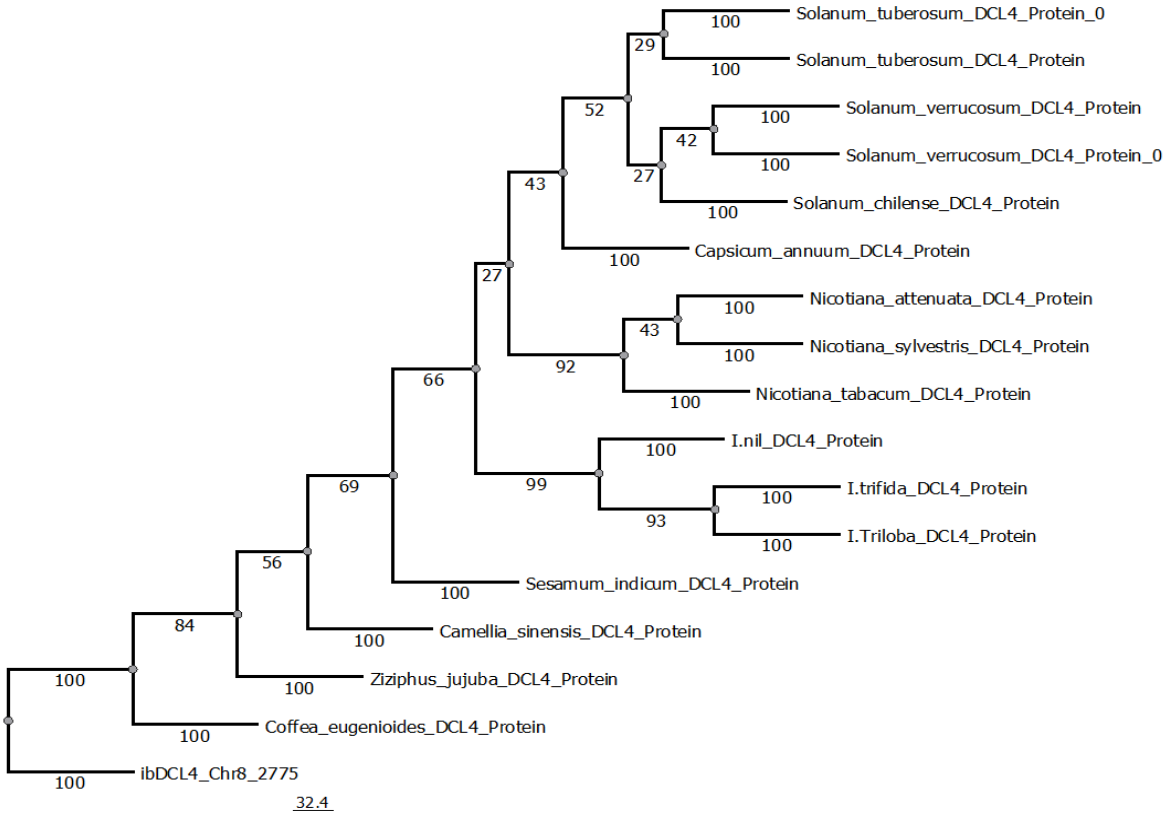
Appendix Figure 5. Phylogenetic tree showing evolutionary relationship of proteins of *Ipomoea batatas* AGO1and AGO1 from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.



Appendix Figure 6. Phylogenetic tree showing evolutionary relationship of proteins of *Ipomoea batatas* DCL1 and DCL1 from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.



Appendix Figure 7. Phylogenetic tree showing evolutionary relationship of proteins of *Ipomoea batatas* DCL2 and DCL2 from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.



Appendix Figure 8. Phylogenetic tree showing evolutionary relationship of proteins of *Ipomoea batatas* DCL4 and DCL4 from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.

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Prevalence and antimicrobial susceptibility of the borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) strains in Bamako, Mali

Dicko O. A.[1], Maïga A.[1,2], Diarra B.[2,3] and Maïga I. I.[1,2]*

[1]Laboratoire de Biologie Médicale et Hygiène Hospitalière du CHU du Point G, Bamako, Mali.
[2]Faculté de Médecine et d'Odontostomatologie de Bamako (FMOS), Université des Sciences, des Techniques et des Technologies de Bamako (USTTB), Bamako, Mali.
[3]Centre Universitaire de Recherche Clinique (UCRC), USTTB, Bamako, Mali.

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The borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) strains have a low-level resistance to penicillinase-resistant penicillins. The aim of this study was to study the prevalence and the antimicrobial susceptibility of BORSA strains in Bamako. A retrospective study was conducted at the University Teaching Hospital of the Point G in Bamako. The *S. aureus* strains were isolated on Columbia agar supplemented with 5% sheep blood, nalidixic acid and colistin. Antimicrobial susceptibility testing was performed using the disc diffusion method on Mueller-Hinton agar. β-lactamase production was determined by nitrocephin disc. Among 735 nonrepetitive strains of *S. aureus*, 41 (5.6%) were BORSA and 335 (45.6%) methicillin-resistant *S. aureus* (MRSA). The prevalence of BORSA strains was 25 (4.9%) and 16 (7.2%) in hospital and out-patients areas, respectively. Of the 41 BORSA strains, five were not β-lactamase producers. Cefoxitin (100%), cephalothin (97.6%), gentamycin (90.2%), amikacin (90.2%), netilmicin (87.8%), pristinamycin (87.8%), tobramycin (82.9%), fusidic acid (83%), lincomycin (78%), amoxicillin combined with clavulanic acid (73.2%), sulfonamides (73.2%), kanamycin (73.2%) fosfomycin (73%) and chloramphenicol (70.7%) were the most active against the BORSA strains. The prevalence of BORSA strains was not high in this study, and the strains were susceptible to a great range of antibiotics.

Key words: Antimicrobial susceptibility, borderline oxacillin-resistant *Staphylococcus aureus*, prevalence, Bamako (Mali).

INTRODUCTION

Staphylococcus aureus belongs to the Staphylococcaceae family (Foster and Geoghegan, 2015). The *S. aureus* infections appear under various clinical aspects such as urinary tract infections, abscesses, septicemia, vaginitis, and/or pleuropulmonary infections (Avril et al., 2000). The borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) has a low level of resistance to penicillin resistant to penicillinases which are oxacillin, cloxacillin and methicillin, that is, antibiotics which are not hydrolyzed by staphylococcal β-lactamases (Hryniewicz

*Corresponding author. E-mail: ibraizet@gmail.com. Tel: +223 76081707.

and Garbacz, 2017). β-lactams are the antibiotics of choice in the treatment of staphylococcal infections (Hryniecz and Garbacz, 2017).

The resistance of the BORSA strains is typically linked to hyperproduction of β-lactamases or in some cases to other mechanisms which are the production of an inducible methicillinase with plasmid mediation, the modification of genes of proteins binding penicillin (PBP) (Cros et al., 2010; Hryniecz and Garbacz, 2017; Maalej et al., 2012). The activity of oxacillin on the BORSA strains is restored by β-lactamase inhibitors such as clavulanic acid or sulbactam (Hryniecz and Garbacz, 2017; Maalej et al., 2012). β-lactamase inhibitors do not restore oxacillin activity when altered proteins binding penicillin occurs (Hryniecz and Garbacz, 2017).

The resistance to methicillin, on the contrary, is due to the acquisition of a new PBP, the PBP2a or PBP2' encoded by chromosomal genes *mecA* or *mecC* (mecALGA251) (Hryniecz and Garbacz, 2017; Maïga et al., 2017). In addition, the BORSA strains are not classified as either methicillin-resistant *S. aureus* strains or methicillin-susceptible *S. aureus* strains (Hryniecz and Garbacz, 2017). The methicillin resistance is crossed between penicillins and cephalosporins (Fleurette, 1989). The BORSA strains, also called methicillin-resistant borderline *S. aureus*, do not have PBP2a or PBP2' (Montanari et al., 1990). The BORSA has been described in Tunisia (Maalej et al., 2012).

In Mali, to the best of our knowledge, no study has been focused on the borderline-oxacillin resistant *Staphylococcus aureus* stains. Thus, the aim of our study was to determine the prevalence and susceptibility to antibiotics of the BORSA strains in Bamako both for epidemiological and clinical purposes.

MATERIALS AND METHODS

Study design and settings

A retrospective study was conducted between January 2014 and December 2020 at the medical biology and hospital hygiene laboratory of the University Teaching Hospital (UTH) of the Point G, Bamako Mali. The UTH of the Point G is the third-pyramidal reference in Mali, and has 522 beds divided between the surgical, intensive care and medical departments.

Bacterial strains

Patients admitted to the different departments of the UTH of the Point G, were hospitalized patients (in-patients), those who were not hospitalized and coming directly to the laboratory for different reasons were called out-patients.

The specimens collected were mainly respiratory origin, urine, pus, blood cultures, and/or vaginal secretions (Table 1).

The isolation of *S. aureus* strains was carried out on Columbia agar supplemented with nalidixic acid (15 mg/l), colistin (10 mg/l) and sheep blood (5%) at 37°C at the oven.

Given the retrospective study design, we included in this study, the *S. aureus* strains with complete drug susceptibility results with additional details of sampling source and date. On the other hand, the *S. aureus* strains without information of the sampling source and/or origin were not included. In this study hospital strains refer to strains isolated from hospitalized patients at the UTH of the Point G, and extra-hospital strains those from out-patients (non-hospitalized patients).

While the molecular methods for identification are rapid and could save more time and labor, they are quite expensive and not implemented in our laboratory yet. Thus, the identification of the strains of *S. aureus* was made by the combination of Gram staining (bioMérieux, France), the presence of a catalase (bioMérieux, France), coagulase (Bio-Rad, France), the Pastorex reagent Staph Plus (Bio-Rad, France) and the API STAPH gallery (bioMérieux, France).

Susceptibility to antibiotics test

The antimicrobial susceptibility testing was carried out on Mueller-Hinton agar (Bio-Rad, France) by the disc method (Agar diffusion method) (Kahlmeter and Turnidge, 2012). The strains of *S. aureus* were classified as "susceptible", "intermediate" or "resistant" according to the recommendations of the Antibiotic Committee of the French Society of Microbiology/European Committee on Antimicrobial Susceptibility Testing in 2015 (CA-SFM/EUCAST) (Amara et al., 2022; Bonnet et al., 2010). For the purpose of analysis, the strains of *S. aureus* classified as <<intermediate>> to the antibiotics tested were considered resistant.

Laboratory procedure

The antimicrobial susceptibility testing was performed on Mueller-Hinton agar poured in a Petri dish. Five to eight colonies identical and isolated from an 18 to 24 h culture of *S. aureus* were suspended in a 5 ml of sterile saline solution which was calibrated to 0.5 McFarland. One milliliter of this suspension was then added to 9 ml of sterile distilled water (dilution 1:10). This second suspension is poured over the entire surface of the Mueller-Hinton agar poured into a Petri dish. The excess is poured into bleach. The seeded agar is left to dry for 15 min at 37°C inside the incubator. Please kindly note that the incubator is not used to dry seeded agar plate. Instead, the plates are allowed to stand on the bench for a while, before the antibiotics dics are introduced. After the seeded agar was dried, the blotting paper discs impregnated with the antibiotic discs to be tested are placed on the surface of the agar using a disc dispenser according to manufacturer's instructions (Bio-Rad, France).

After a first diffusion of the antibiotics at 30 min at room temperature, the Petri dish is incubated at 37°C for 18 to 24 h, in the inverted position (cover down). The paper disc impregnated with oxacillin is placed on the surface of another seeded agar in Petri dish (diameter 90 mm) and placed in the incubator at 30°C for 24 to 48 h in the inverted position also. The reading is performed in measuring the diameter of inhibition of each antibiotic disc using a caliper in contact of growth.

Antibacterial agents tested

The antibiotics tested are penicillin G (6 µg), cephalothin (30 µg), oxacillin (5 µg), amoxicillin combinated with clavulanic acid (20 µg + 10 µg), cefoxitin (30 µg), gentamycin (10 UI), kanamycin (30 UI), tobramycin (30 µg), netilmicin (30 µg), amikacin (30 µg), streptomycin (10 µg), erythromycin (15 µg), lincomycin (15 µg), pristinamycin (15 µg), chloramphenicol (30 µg), tetracycline (30 µg), sulfonamides (200 µg), trimethoprim(5 µg), norfloxacin (5 µg),

Table 1. Distribution of 735 strains of *Staphylococcus aureus* according to the origin and the specimens.

Specimen	Hospital strains (No. of strains)	Extra-hospital strains (No. of strains)	Total (Rate in %)
Urines	196	165	361 (49.1)
Pus	186	29	215 (29.2)
Blood cultures	106	0	106 (14.4)
Vaginal secretions	0	18	18 (2.5)
Pleurisy	15	0	15 (2.4)
Catheters	6	5	11 (1.5)
Sputum	1	3	4 (<1)
Ascites	3	0	3 (<1)
Prostatic fluid	0	2	2 (<1)
Total	513	222	735 (100)

Source: Authors

fosfomycin (50 µg) and fusidic acid (10 µg) (Bio-Rad, France).

β-lactams resistance phenotypes

While the *S. aureus* strains susceptible to cefoxitin and oxacillin were considered methicillin-susceptible *S. aureus* (MSSA), those resistant to cefoxitin and oxacillin have been considered methicillin-resistant *Staphylococcus aureus* (MRSA) (Cattoir and Leclercq, 2012; Mougeot et al., 2001). In addition, the *S. aureus* strains resistant or intermediate to oxacillin and susceptible to cefoxitin were considered as borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) (Hryniewicz and Garbacz, 2017). Moreover, the BORSA strains resistant or intermediate to penicillin G produced β-lactamase. The production of β-lactamase was confirmed using the nitrocephine (cefinase) disc (bioMérieux, France). The penicillin G-susceptible BORSA strains with a fuzzy border were not β-lactamase producers. We compared the susceptibility of the BORSA strains to that of randomly selected MSSA strains.

Ethical consideration

The use of the results of the susceptibility to antibiotics test of isolated *S. aureus* strains was done after patients signed an informed consent. The results were de-identify with anonymous numbers, and the publication of the results was made with the permission of the leadership of the Point G's UTH.

Statistical analysis

The results of the susceptibility to antibiotics were used to monitor the bacterial resistance's profile at the Point-G's UTH and Bamako.

Data entry and computer processing were done using Epi Info version 7.1 software. The χ^2 test was used to compare our proportions with a significance level of $p \leq 0.05$.

RESULTS

Global characteristics of patients and isolates

In total, 735 non-repetitive strains of *S. aureus* were isolated from 734 persons between January 2014 and December 2020 and included in the study. The mean age of patients was 47.61 ± 19.17 years with extremes of three and 98 years. The sex ratio (male/female) of patients was 0.94. They were in 513 (69.8%) of hospital strains and 222 (30.2%) out-patients' strains.

The annual frequency of the *S. aureus* strains during the study period is presented in Figure 1.

Frequency of BORSA within *S. aureus* strains

Of the 513 hospital strains of *S. aureus*, 25 (4.9%) were BORSA, 243 (47.4%) MRSA and 245 (47.8%) MSSA. At the same time, of 222 out-patients' strains, 16 (7.2%) were BORSA, 92 (41.4%) MRSA and 114 (51.4%) MSSA. Thus, the BORSA strains prevalence was independent of origin (p= 0.18).

The distribution of the 735 strains according to the phenotype of resistance to β-lactams and the sample is reported in Table 2. The prevalence of the BORSA strains was independent of the sampling sites. Moreover, the prevalence of the MRSA strains was higher in the urine than in the other samples ($p < 10^{-6}$).

The bacteriological characteristics of the BORSA hospital and extra-hospital strains are reported in Table 3. Two out-patients' BORSA strains were isolated from urine in the same patient in April 2017: one of them had diminished susceptibility to norfloxacin, chloramphenicol and streptomycin.

Moreover, of the 25 BORSA hospital strains, four did not produce β-lactamase, and of 16 BORSA extra-hospital strains, one did not produce β-lactamase.

Susceptibility profile of S. aureus strains

All our BORSA strains were resistant to oxacillin, but susceptible to cefoxitin. In addition, 40 (97.6%) were

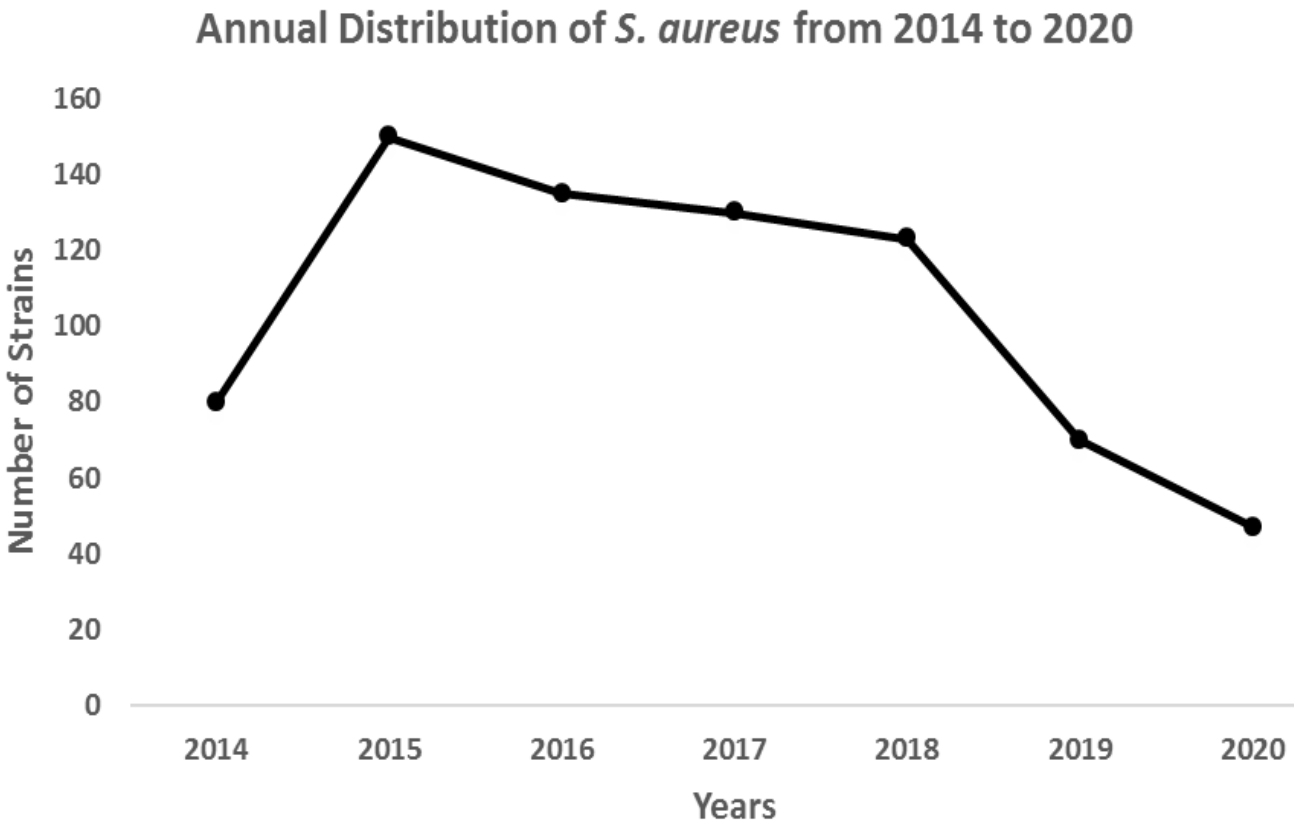

Figure 1. Annual distribution of the *Staphylococcus aureus* strains from 2014 to 2020 at the Point G University Teaching Hospital.
Source: Authors

Table 2. Distribution of 735 strains of *Staphylococcus aureus* according to the phenotype and the specimens.

Specimen	BORSA [n (%)]	MRSA [n (%)]	MSSA [n (%)]	Total [n (%)]
Urines	13 (3.6)	223 (61.8)	125 (34.6)	361 (100)
Pus	17 (7.9)	54 (25.1)	144 (67)	215 (100)
Blood cultures	7 (6.6)	41 (38.7)	58 (54.7)	106 (100)
Vaginal secretions	1	6	11	18
Pleurisy	1	1	13	15
Catheters	2	6	3	11
Sputums	0	3	1	4
Ascites	0	0	3	3
Prostatic fluid	0	1	1	2
Total	41	335	359	735
	5.6	45.6	48.8	100

$\chi^2 = 72.76$; d.d.l. = 1; $P < 10^{-6}$. BORSA = borderline oxacillin-resistant *S. aureus*; M RSA= methicillin-resistant *S. aureus*; M SSA= methicillin-susceptible *S. aureus*.
Source: Authors

susceptible to cephalothin, 30 (73.2%) to the combination of amoxicillin + clavulanic acid, and five (12.2%) to the penicillin G. Among the 36 BORSA strains producing β-lactamase, 25 were susceptible to the combination amoxicillin + clavulanic acid and 11 were resistant.

The comparative antibiotic susceptibility of the BORSA and MSSA strains is reported in Table 4. The most active antibiotics on the BORSA strains were cefoxitin, cephalothin, gentamycin, amikacin, netilmicin, amoxicillin + clavulanic acid association, tobramycin, pristinamycin,

Table 3. Bacteriological characteristics of 41 borderline-oxacillin resistant *Staphylococcus aureus* hospital and extra-hospital strains production.

Date	Specimens	Departments	Diameter zone inhibition (mm)					β-lactamase
			OXA	P	AMC	CF	FOX	
1/17/2015	Pus	Surgery B	19	30	35	36	31	Negative
2/8/2015	Pus	Int med.	16	33	30	32	30	Negative
4/27/2015	Pus	Surgery B	0	0	19	21	27	Positive
5/16/2015	Pus	HMO	13	10	17	23	25	Positive
6/14/2015	Pus	Surgery B	18	38	44	36	32	Negative
8/29/2015	Blood culture	HMO	15	21	25	15	26	Positive
10/5/2015	Urine	Int med.	18	12	23	32	27	Positive
12/4/2015	Blood culture	Int med.	16	14	30	28	26	Positive
12/27/2015	Blood culture	Emergencies	15	10	25	27	26	Positive
1/9/2016	Blood culture	Emergencies	15	11	26	27	27	Positive
1/31/2016	Urine	Neurology	15	27	29	33	29	Positive
4/5/2016	Blood culture	Emergencies	18	0	23	25	28	Positive
1/7/2017	Pus	Emergencies	18	21	30	35	28	Positive
1/29/2017	Pus	Surgery A	14	18	34	40	25	Positive
4/9/2017	Pus	Surgery A	0	14	25	28	28	Positive
6/9/2017	Pus	Surgery B	17	20	17	23	25	Positive
8/14/2017	Blood culture	Emergencies	12	13	23	29	30	Positive
8/25/2017	Pus	Int med.	14	16	26	31	32	Positive
9/23/2018	Pus	Surgery A	0	16	23	28	29	Positive
9/29/2018	Pus	Gynecology	0	10	20	27	30	Positive
10/6/2018	Pus	Inf Dis	18	33	40	29	33	Negative
9/17/2019	Pus	Surgery B	0	0	20	25	26	Positive
9/20/2019	Pleurisy	Emergencies	0	0	20	23	25	Positive
9/27/2019	Blood culture	Int med.	0	0	17	27	28	Positive
1/28/2020	Urine	ICU	0	0	23	22	25	Positive
3/20/2015	Urine	EHS	0	0	24	30	28	Positive
6/9/2015	Urines	EHS	18	35	33	36	26	Negative
12/28/2016	Pus	EHS	17	22	35	35	30	Positive
4/2/2017	Pus	EHS	14	24	30	33	28	Positive
4/9/2017	Urine	EHS	0	11	23	29	25	Positive*
4/9/2017	Urine	EHS	0	12	23	29	27	Positive
4/15/2017	VS	EHS	15	15	29	32	30	Positive
7/15/2017	Urine	EHS	0	24	34	20	32	Positive
7/24/2017	Urine	EHS	0	0	21	30	33	Positive
8/12/2017	Urine	EHS	16	19	30	34	26	Positive
9/16/2017	Urine	EHS	17	20	29	19	30	Positive
11/11/2017	Urine	EHS	18	22	30	32	27	Positive
12/19/2017	Urine	EHS	0	0	29	34	30	Positive
6/13/2019	Catheter	EHS	19	0	17	25	28	Positive
6/20/2019	Pus	EHS	18	8	20	27	28	Positive
5/25/2020	Catheter	EHS	0	0	18	30	29	Positive

VS = Vaginal secretions; HMO = hematology-medical oncology; Int med = internal medicine; Inf Dis= Infectious Diseases; ICU = intensive care unit; EHS = extra-hospital strains; OXA = oxacillin; P = penicillin G; AMC = amoxicillin combined with clavulanic acid; CF = cephalothin; FOX = cefoxitin. *Diminished susceptibility to norfloxacin, chloramphenicol and streptomycin.
Source: Authors

fusidic acid, lincomycin, chloramphenicol, sulfonamides, kanamycin, and fosfomycin. Moreover, MSSA strains were more susceptible to amoxicillin + clavulanic acid, kanamycin, tobramycin, erythromycin, lincomycin,

Table 4. Comparative antimicrobial susceptibility of BORSA and MSSA strains.

Antibiotics	BORSA (No. of strains = 41)		MSSA (No. of strains = 82)		P-value
	S [n (%)]	I+R [n (%)]	S [n (%)]	I+R [n (%)]	
Penicillin G	5 (12.2)	36 (87.8)	16 (19.5)	66 (80.5)	0.4
AMC	30 (73.2)	11 (26.8)	82 (100)	0 (0)	0.001
Oxacillin	0 (0)	41 (100)	82 (100)	0 (0)	
Cephalothin	40 (97.6)	1 (2.4)	82 (100)	0 (0)	0.72
Gentamycin	37 (90.2)	4 (9.8)	82 (100)	0 (0)	0.02
Kanamycin	30 (73.2)	11 (26.8)	75 (91.5)	7 (8.5)	0.015
Tobramycin	34 (82.9)	7 (17.1)	81 (98.8)	1 (1.2)	0.0029
Amikacin	37 (90.2)	4 (9.8)	82 (100)	0 (0)	0.019
Netilmicin	36 (87.8)	5 (12.2)	82 (100)	0 (0)	0.006
Streptomycin	25 (61)	16 (39)	56 (68.3)	26 (31.7)	0.54
Erythromycin	24 (58.5)	17 (41.5)	72 (87.8)	10 (12.2)	0.001
Lincomycin	32 (78)	9 (22)	82 (100)	0 (0)	0.001
Pristinamycin	36 (87.8)	5 (12.2)	82 (100)	0 (0)	0.006
Norfloxacin	24 (58.5)	17 (41.5)	63 (76.8)	19 (23.2)	0.058
Chloramphenicol	29 (70.7)	12 (29.3)	75 (91.5)	7 (18.5)	0.006
Tetracycline	16 (39)	25 (61)	35 (42.7)	47 (57.3)	0.846
Sulfonamides	30 (73.2)	11 (26.8)	69 (84.2)	13 (15.8)	0.23
Trimethoprim	25 (61)	16 (39)	60 (73.2)	22 (26.8)	0.24
Fusidic acid	34 (83)	7 (17)	79 (96.3)	3 (3.7)	0.0267
Fosfomycin	30 (73.2)	11 (26.8)	81 (98.8)	1(1.2)	0.001

BORSA = borderline oxacillin-resistant *S. aureus*; MSSA = methicillin-susceptible *S. aureus*; AMC = amoxicillin combined with clavulanic acid; S = susceptible; I = intermediate; R = resistant; P = probability.
Source: Authors

pristinamycin, chloramphenicol, fusidic acid and fosfomycin the BORSA strains: the differences are statistically significant.

DISCUSSION

In this study, we determine the prevalence and susceptibility to antibiotics of the BORSA strains between 2014 and 2020, using the disk method (agar diffusion technique): an international reference technique (Kahlmeter and Turnidge, 2012). To the best of our knowledge, this study is the first study of its kind conducted in Bamako, Mali making difficult comparison of previous data from Mali. The identification of our strains was based on their morphological, physiological and biochemical characteristics (Avril et al., 2000; Fleurette, 1989). The identification of *S. aureus* was characterized by its susceptibility to lysostaphin, nitrofurantoin and its resistance to the vibriostatic compound O/129 and to bacitracin (Fleurette, 1989).

The results of the antibiogram were interpreted in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology and the European Committee on Antimicrobial Susceptibility Testing (Amara et al., 2022; Bonnet et al., 2010).

Other higher sensitivity and specificity methods were used for the detection of resistance to methicillin such as the oxacillin disc placed either on a Petri-dish of Mueller-Hinton agar incubated between 25 and 30°C and observed after 24 and 48 h or on a Petri-dish of hypersalted Mueller-Hinton agar incubated at 37°C, the use of probes recognizing *mec* genes, and the agglutination reactions of latex particles sensitized with anti-PBP2a antibodies (April et al., 2000). But the agglutination reactions of latex particles sensitized with anti-PBP2a antibodies is not routinely used (Maalej et al., 2012).

Currently, the identification of the MRSA and BORSA strains are based on the use of oxacillin and cefoxitin discs on Mueller-Hinton agar incubated at 37°C and examined 24 h later. For oxacillin, the interpretation must be done within 48 h if there is a low amount of culture (Bonnet et al., 2010; Cattoir and Leclercq, 2012). The use of the cefoxitin disc alone could make it possible to wrongly classify the BORSA strains among those with MSSA. The use of cefoxitin (30 µg) and moxalactam (30 µg) discs make it possible to identify MRSA at the Saint-Louis Hospital in France with excellent sensitivity (Felten et al., 2002).

In this study, the BORSA strains prevalence was 5.6%

and independent of the sampling site and the origin of the strains. The MRSA strains prevalence was 46.2% and was higher in the urine than in the other samples (Table 2). As observed in Myanmar (Soe et al., 2021), this high prevalence in urine samples, may have occurred because of urinary catheterization practice and the colonization by MRSA of indwelling urinary catheters. In Tunisia, of the 1,895 strains of *S. aureus* isolated between 2006 and 2011 at the University Hospital of Sfax, 451 (21.9%) were MRSA and 23 (1.2%) BORSA (Maalej et al., 2012). These rates are lower than ours. In a previous study conducted in 2007 in our department, the frequency of MRSA strains was 50.3 and 50.8% among hospital and out-patients' settings, respectively (Tchougoune, 2007). Those rates are closed to these current findings. Usually, the prevalence of the BORSA varies from 1.4 to 12.5% (Hrynievicz and Garbacz, 2017), and our rate is within the intermediate values (Table 2).

In this study, we isolated the BORSA strains from different collection sites, and we did not find a correlation between the strains and the site of collection (Table 2). This was the same in Sfax (Tunisia), and in Toronto (Canada) (Maalej et al., 2012; Leahy et al., 2011). Moreover, the hospital BORSA strains were coming from different units, and it was the same in Tunisia and Canada. In addition, we did not observe an outbreak of the BORSA strains during the study period, and this was the same in Tunisa as well (Maalej et al., 2012). Moreover, when analysis the 16 BORSA strains isolated from children with cystic fibrosis by pulsed-field restricted DNA electrophoresis in Toronto: no link was observed between the strains (Leahy et al., 2011). No link between the 23 strains was observed in Tunisa (Maalej et al., 2012).

Of the 41 BORSA strains, five did not produce β-lactamase (Table 3), and this was the same in Canada, where out of 16, four did not produce β-lactamase (Leahy et al., 2011). At the same time in Tunisia, all the BORSA strains produced β-lactamase (Maalej et al., 2012). This could probably be explained by the sample size which was different in the three countries.

In contrast to Tunisia and Canada (Leahy et al., 2011; Maalej et al., 2012), the combination of amoxicillin + clavulanic acid was not active on 11 BORSA strains out of 41 (Tables 3 and 4). This is probably explained by other mechanisms (Croes et al., 2010; Hryniewicz and Garbacz, 2017; Jupeau-Vessières and Scavizzi, 1994; Maalej et al., 2012). Moreover, both BORSA strains in Mali and Tunisia were susceptible to cefoxitin (Table 3) (Maalej et al., 2012).

In addition, penicillin G, cephalothin, streptomycin, norfloxacin, tetracycline, sulfonamides and trimethoprim were not more active on MSSA strains than on BORSA strains (Table 4). Gentamycin (90.2%), amikacin (90.2%), tobramycin (82.9%), netilmicin (87.8%) and kanamycin (73.2%) were the most active aminoglycosides on our BORSA strains (Table 4). Among the BORSA strains isolated in Sfax, 13 out of 23 strains were susceptible to kanamycin, 20 to gentamycin and 19 to tobramycin. Fusidic acid (83%), fosfomycin (73%) and chloramphenicol (70.7%) had good activity on our BORSA strains (Table 4). All the BORSA strains isolated in Sfax were susceptible to fosfomycin, chloramphenicol and glycopeptides (Maalej et al., 2012). Among our BORSA strains, one out of two strains was susceptible to erythromycin (Table 4). Eleven out of 23 BORSA strains were susceptible to erythromycin in Sfax, Tunisia (Maalej et al., 2012). Thirteen BORSA strains out of 16 were susceptible to erythromycin in Toronto, Canada (Leahy et al., 2011).

Thus, the search of BORSA and MRSA strains is a priority at the UTH of the Point G in Bamako.

Our study has some limitations including the retrospective approach which may have precluded to firmed conclusion because of the loss of sensitive information. In addition, we have more patients who were not included due to missing data either on sample types and origins or culture results and have used patients who were diagnosed in urban Bamako district only who may have different results from rural areas of Mali.

Despite these limitations, we studied a relatively high sample size to determine the prevalence and the antimicrobial susceptibility of the borderline oxacillin-resistant *Staphylococcus aureus* strains in Bamako in sample types. In addition, given that almost all the regions in Mali are getting a bacterial culture system, thus, a larger sample size and sampling from all the different regions of Mali would be essential for obtaining a true country profile of BORSA and MRSA phenotypes.

Conclusion

The BORSA strains prevalence is not high in Bamako and was not associated with the origin and sampling site. The BORSA strains were susceptible to many molecules: cefoxitin, cephalothin, gentamycin, amikacin, netilmicin, tobramycin, the combination amoxicillin + clavulanic acid, pristinamycin, acid fusidic, lincomycin, chloramphenicol, sulfonamides, kanamycin, and fosfomycin. In contrast, antibiotics such as erythromycin, norfloxacin, tetracycline, trimethoprim, and streptomycin should not be used in the treatment of infections caused by strains of the BORSA strains in Bamako.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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