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Bacteriological Profile of Suppurative Infections at the University Departmental Hospital of Borgou/Alibori in Benin

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Abstract: Purulent infections are common and can be caused by a variety of microorganisms. The present study focuses on 216 purulent samples and aims to determine their bacteriological profile at the Clinical Biology Department of the Borgou and Alibori Departmental Hospital. Isolation and identification of microorganisms and antibiotic susceptibility testing were performed using standard microbiological techniques. Sixty-one of the 100 participants (61%) were male. Participants ranged in age from 8 months to 76 years. The most common age group was between 50 and 60 years (22.70%). The prevalence of purulent infection in the study population was 43.52%, with a predominance of monomicrobial cultures (79%). Cytobacteriological examination of the pus showed that of 114 strains isolated responsible for purulent infection, 53 (46.50%) were gram-negative bacteria compared with 61 (53.50%) gram-positive. The most frequently isolated species were: *Staphylococcus aureus* (47.37%), *Escherichia coli* (14.04%), *Klebsiella pneumoniae* (7%). Analysis of the antibiogram results for the isolated strains revealed isolates resistant to the main antibiotic families. Multi-resistant strains such as MRSA (Methicillin-Resistant *Staphylococcus aureus*) and ESBL (Extended Spectrum Beta-Lactamase) producing strains were identified. Antibiotic resistance is a real public health problem that can lead to the death of infected patients due to the lack of appropriate drugs for better management, hence the importance of controlling and rationalising medical prescribing in the event of infection.

Keywords: *Infection, suppuration, bacteria, antibiotics, resistance*

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1. Introduction

Suppurative infections are characterised by severe local inflammation with production of pus (whitish or yellowish fluid) consisting of leukocytes, cellular debris and necrotic tissue [1]. It is an invasion and multiplication of pathogenic microorganisms in a part of the body that can lead to tissue damage [2]. These infections can be superficial (skin infections) or deep (internal organs) [3]. The microorganisms responsible for inflammation and suppuration are pyogenic bacteria [2]. In most studies, the majority of isolates are aerobic and include gram-positive cocci (*Staphylococcus aureus*, *Streptococcus pyogenes*, Enterococcus) and gram-negative bacilli (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus and Pseudomonas*) [4]. Antibiotics, molecules capable of inhibiting the growth or elimination of bacteria, represent one of the most important discoveries in medicine and have saved millions of lives every year since the 1940. However, their excessive and sometimes inappropriate use has led to the emergence of antibiotic-resistant bacteria, which in turn pose a major challenge to the proper management of infected patients. The emergence of multi-resistant strains leads to prolonged illness, higher healthcare costs and an increased risk of death from infection. Suppurative infections are still common in developing countries and treatment remains a significant challenge despite advances in microbiological techniques, antibiotics and surgical treatments [2]. To ensure appropriate and effective therapy, it is necessary to identify and treat the site of inflammation. This is the context of the present study, which aims to contribute to the fight against antimicrobial resistance by establishing the bacteriological profile of suppurative infections and the reactivity of isolated bacteria to conventional antibiotics.

2. Materials and Reagents

The following equipment and reagents were used: microscope, oven, culture media (blood agar, methylene blue-eosin agar, colimycin-nalidixic acid agar and Chapman's agar), stains (gentian violet, Lugol's, alcohol, fuschin, etc.) and antibiotic discs (amoxicillin/clavulanic acid, ampicillin, ceftazin, cefoxitin, ceftriazone, cotriazone, etc.).

3. Methodology

The colour (yellow-green to reddish-brown), consistency (cloudy, very thick and sticky) and odour of the pus samples were noted on macroscopic examination. In the Examination in the fresh state, the mobility and abundance of germs were observed at x40. Cultures were grown on agar to obtain distinct, well-separated colonies after plating using the dial technique and incubation at 37°C for 24 hours under aerobic (methylene blue eosin, Chapman) and anaerobic (blood agar and nalidixic acid-colimycin) conditions. For Gram staining, each sample was spread, dried and fixed, then stained with gentian violet, Lugol's, bleached with alcohol and finally restained with fuchsin. Microscopic observation was carried out using a 100 objective and immersion oil. A double stain is used to determine the shape, arrangement (assembly method), purity and biochemical nature of the microorganism wall. This stain is used to classify bacteria according to their ability to bind crystal violet. Testing for catalase has allowed the differential diagnosis of Staphylococcus and Streptococcus, which are Gram-positive cocci. This enzyme breaks down hydrogen peroxide into water and oxygen gas. The oxidase test allowed us to make a differential diagnosis between non-oxidative Gram-negative bacilli (Enterobactericeae and Acinetobacter) and oxidative Gram-negative bacilli (Pseudomonas, Pasteurella, etc.). The Api 20 E gallery was used to identify gram-negative bacilli. The Muller Hinton agar diffusion method was used to test the susceptibility of the isolated micro-organisms to conventional antibiotics.

4. Results

√ Sociodemographic results

The figure below shows the breakdown of participants by age.

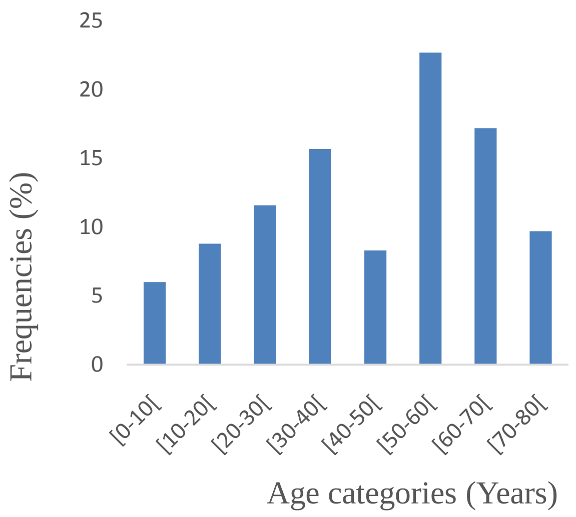

Figure 1. Age distribution of participants

According to this figure, participants aged between 50 and 60 years are the most represented with a percentage of 22.70%.

The breakdown of participants by gender is shown in Figure 2.

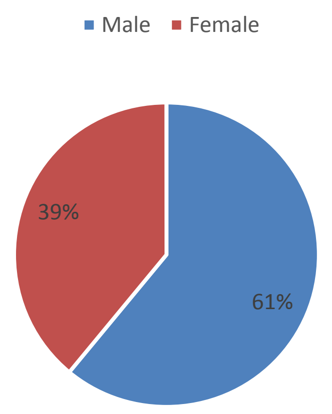

Figure 2. Breakdown of participants by gender

According to the breakdown of participants by gender, males were the most represented at 61%.

√ Prevalence of purulent infections

Of the 216 pus specimens obtained, 94 were positive, or 43.52% of cases, compared with 122 negative specimens, or 56.48% (Table 1).

Table 1. Prevalence of purulent infections

	Positive	Negative	Total
Effectif	94	122	216
Frequenies (%)	43.52	56.48	100

√ Distribution of samples by culture type

The figure below shows the culture type.

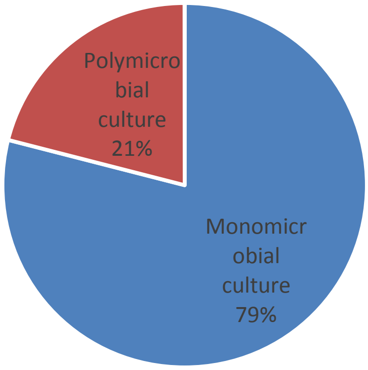

Figure 3. Frequency (%) of positive results in patients by type of culture

Cytobacteriological analysis of the pus allowed us to divide the samples into two groups: monomicrobial cultures with a rate of 79% and polymicrobial cultures with a rate of 21% (Figure 3).

√ Distribution of samples by Gram stain and bacterial group

A total of 114 strains were isolated from 94 positive purulent samples, representing 21 different species. The isolation rates for gram-negative bacilli and gram-positive cocci were 46.5% and 53.5%, respectively. In this distribution, Staphylococcus strains were the most dominant, followed by Enterobacteriaceae (Table 2).

√ Distribution of positive samples by Gram stain and bacterial species isolated

Table 3 shows that *Staphylococcus aureus* was the most frequently isolated species with 54 strains representing 47.37% of isolates, followed by *Escherichia coli* with 16 strains (14.04%) and *Pseudomonas aeruginosa* with 6 strains (05.26%).

Table 2. Distribution by Gram stain and bacterial group

Germes	Effectifs	Frequencies (%)	Groupe	Effectifs	Frequencies (%)
Gram-negative bacilli	53	46.5	Enterobacteriaceae	46	40.35
			Gram negative bacilli, non-fermentative	07	06.14
Gram-positive cocci	61	53.5	*Staphylococcus sp*	54	47.37
			Streptococcus sp	05	04.39
			Enterococcus sp	02	01.5
Total	**114**	**100**		114	100

Table 3. Distribution of the main bacterial strains isolated

Families/Genus	Frequencies (%)	Species		Effectifs
Enterobacteriaceae (46)	40.35%	*Escherichia coli*		**16**
		Aeromonas hydrophila		**2**
		Salmonella spp		**1**
		Myroides/Chyseobacters		**1**
		Yersinia spp		**1**
		Kluyvera spp		**3**
		Klebsiella	*K. pneumoniae*	**4**
			K. terrugena	**2**
			K.ornithilonitica	**2**
		Enterobacter	*E. Aerogenes*	**2**
			E. cloacae	**4**
		Serratia	*S. Odorifer*	**2**
			S. fecaria	**1**
		Citrobacters	*C. braaki*	**2**
			C. freundi	**2**
		Proteus	*P. vulgaris*	**1**
Gram negative bacilli, non-fermentative (07)	06.14%	*Pseudomonas aeruginosa*		**6**
		Acinetobacter baumannii		**1**
Enterococcus (02)	01.75%	*Enterococcus faecalis*		**2**
Streptococcus (05)	04.39%	*Streptococcus pyogenes*		**5**
Staphylococcus (54)	47.37%	*Staphylococcus aureus*		**54**

√ Antibiotic resistance

• Resistance profile of *Escherichia coli* (n=16)

Antibiotic susceptibility testing of the 16 isolated *Escherichia coli* strains showed variable results. The rate of antibiotic resistance is shown in the table below.

• Resistance profile of *Klebsiella spp* (n=8)

The antibiogram results for the 8 *Klebsiella spp* strains are shown in Table 5. The resistance rate was 75% for amoxicillin/clavulanic acid and 50% for ampicillin, penicillin and gentamicin.

Table 4. Resistance profile of *Escherichia coli*

Antibiotics	Number of Strains Tested	Number of Resistant Strains	Resistance rate (%)
Amoxicillin/Ac clavulanique	16	15	93.75
Ampicillin	16	11	68.75
Ceftazidine	16	7	43.75
Cefoxitin	16	6	37.5
Ceftriazone	16	7	43.75
Cotrimoxazole	16	9	56.25
Penicillin	16	9	56.25
Gentamicin	16	8	50.00
Ciprofloxacin	16	12	75.00
Aztreonam	16	7	43.75
Tretracycline	16	5	31.25
Vancomycin	16	4	25.00
Chloramphenicol	16	7	43.75

Table 5. Resistance profile of *Klebsiella spp*

Antibiotics	Number of Strains Tested	Number of Resistant Strains	Resistance rate (%)
Amoxicillin/Ac clavulanique	8	6	75.00
Ampicillin	8	4	50.00
Ceftazidine	8	2	25.00
Cefoxitin	8	2	25.00
Oxacillin	8	2	25.00
Cotrimoxazole	8	3	37.50
Penicillin	8	4	50.00
Gentamicin	8	4	50.00
Ciprofloxacin	8	3	37.50
Aztreonam	8	3	37.50
Imipenem	8	2	25.00
Levofloxacin	8	1	12.50
Chloramphenicol	8	2	25.00

- **Enterobacter resistance profile (n=6)**

The 6 Enterobacter strains isolated showed significant resistance to amoxicillin/clavulanic acid (83.3%), ampicillin and penicillin (66.6%), the most widely used antibiotics of the beta-lactam family.

Table 6. Enterobacter resistance profile

Antibiotics	**Number of Strains Tested**	**Number of Resistant Strains**	**Resistance rate (%)**
Amoxicillin/Ac clavulanique	6	5	**83.33**
Ampicillin	6	4	**66.60**
Ceftazidine	6	1	**16.60**
Cefoxitin	6	1	**16.60**
Ceftriazone	6	2	**33.30**
Cotrimoxazole	6	3	**50.00**
Penicillin	6	4	**66.60**
Gentamicin	6	2	**33.30**
Ciprofloxacin	6	3	**50.00**
Aztreonam	6	2	**33.30**
Tretracycline	6	2	**33.30**
Vancomycin	6	1	**16.60**
Chloramphenicol	**6**	**3**	**50.00**

- **Resistance profile of *Pseudomonas aeruginosa* (n=6)**

Strains of *Pseudomonas aeruginosa* continue to be quite susceptible to antibiotics, with the highest resistance rates recorded for piperacillin (33.3%), ticarcillin (16.6%), gentamicin (33.3%) and ciprofloxacin (50%).

Table 7. Resistance profile of *Pseudomonas aeruginosa*

Antibiotics	Number of Strains Tested	Number of Resistant Strains	Resistance rate (%)
Imipenem	6	2	33.30
Ceftazidine	6	1	16.60
Cefoxitin	6	2	33.30
Ticarcillin	6	1	16.60
Gentamicin	6	2	33.30
Ciprofloxacin	6	2	50.00
Piperacillin	6	2	33.30
Fosfomycin	6	2	33.30
Chloramphenicol	6	1	16.60

- **Resistance profile of *Staphylococcus aureus* (n=54)**

Almost 70% of the strains were resistant to penicillin. There were 21 strains resistant to oxacillin (39%). There were also significant rates of resistance to other antibiotics.

Table 8. Resistance profile of *Staphylococcus aureus*

Antibiotics	Number of Strains Tested	Number of Resistant Strains	Resistance rate (%)
Amoxicillin	54	34	62.90
Ampicillin	54	27	50.00
Imipenem	54	22	40.70
Levofloxacin	54	25	46.20
Ceftriazone	54	18	33.30
Cotrimoxazole	54	22	40.70
Penicillin	54	39	72.20
Gentamicin	54	26	48.10
Ciprofloxacin	54	29	53.70
Oxacillin	54	23	42.60
Tetracycline	54	25	46.30
Erytromycin	54	22	40.70
Chloramphenicol	54	19	35.20

5. Discussion

Pyogenic infections are a public health problem. Knowledge of the pathogens responsible for these infections and their susceptibilities is useful for choosing an appropriate antimicrobial treatment, but also for an effective fight against antimicrobial resistance [5]. Between March and June 2024, at the Clinical Biology Department, Bacteriology Section of the Borgou Alibori Departemental Hospital, 94 samples were found positive out of 216 pus samples collected, giving a positivity rate of 43.51%. This result is lower than that reported in a study conducted in India in 2021, which found an overall positivity rate of 85.02% [6]. The results obtained in the present study are lower than those reported by Bankar *et al.* in 2018 [7] (78.55%) and Gomatheswari and Jeyamurugan in 2017 (71.49%) [8]. During the study period and based on the inclusion criteria, there was a male predominance with a frequency of 61% compared to 39% for females (sex ratio M/F= 1.56). These results are lower than those of Hamid *et al.* (2020) [9] in a cross-sectional study in Sudan, who reported male predominance with a sex ratio M/F= 2.73. Macia's team, in 2017 [10], reported a male prevalence of 50.60%, which corresponds to a sex ratio of 1.02. These results also differ from those of Maharjan *et al*., in 2020 [11], who reported a female predominance (52.9%) with a sex ratio M/F= 0.89. In the present study, cytobacteriological analysis of pus allowed us to classify cultures into two groups: mono-microbial cultures with a rate of 79% and polymicrobial cultures with a rate of 21%. These results are consistent with those reported by Verma, (2012) [12] and Amin *et al*, (2017) [1] who found rates of 84.31% and 79.92% of mono-microbial cultures, respectively. Infections are polymicrobial if they are deep and/or chronic and/or already treated with antibiotics. The sampler, instruments, rooms and hospital environment can also be sources of sample contamination. The medical literature reports that suppurative infections are dominated by Gram-positive bacteria [13]. Gram-positive and gram-negative bacteria were isolated from all positive cultures by Gram staining. Cytobacteriological analysis identified 46.5% of Gram-negative bacteria and 53.5% of Gram-positive bacteria. Our results are in agreement with those of Bankar *et al*, (2018) [7] (51.97% Gram-negative and 47.36% Gram-positive) and Sharma *et al*, (2018) [6] (89% Gram-negative and 11% Gram-positive). The distribution of isolated strains according to bacterial groups shows that Staphylococcus is the most isolated pathogen with 47.37% of positive cases (54 strains), followed by Enterobacteriaceae with a rate of 40.35% (46 strains), then non-fermentative Gram-negative bacilli and Streptococcus with a rate of 6.14% (7 strains) and 4.39% (5 strains) respectively, and finally Enterococcus. These results are similar to those found by Chacón-Quesada and colleagues in 2021 [13], where gram-positive cocci, and especially staphylococci, are more common in surgical site infections than other bacterial groups. However, these results are in complete contrast to those of Rahma *et al*., (2021) [14], where Enterobacteriaceae accounted for 70% of the 1291 strains isolated. These results also contradict those obtained by Gomatheswari and Jeyamurugan (2017) [8], who found a predominance of Enterobacteriaceae followed by Gram-positive cocci. *Staphylococcus aureus* was the most isolated bacterial species in the present study (47.37%) followed by *Escherichia coli* (14.04%), *P. aeruginosa* (5.26%), *Streptococcus pyogenes* (4.39%) and *K. pneumoniae* (3.50%). Our results are better than those reported by Amin *et al*, (2017) (14.3%) and Gomatheswari and Jeyamurugan, (2017) [8] (18.5%). *Staphylococcus aureus* is a common human commensal that can colonise the skin, nose and throat with the anterior nares as the main reservoir. It is the most common cause of skin and soft tissue infections. It causes a wide variety of infections, ranging from benign (impetigo) to immediate death. It's the most common pathogen isolated from surgical site infections, skin abscesses and purulent cellulitis. However, the results of the present study differ from those of Kanakadurgamba *et al.*, 2021 [15] who reported that *E. coli* and *Klebsiella pneumoniae* were the predominant isolates, followed by *Pseudomonas aeruginosa* and *S. aureus*. Although *E. coli* is in second place in the present work, it is still relatively low compared to its predominance reported in the literature by Le Turnier's team in 2017 [16]. Antibiotic resistance is a growing global concern. The evolution of bacteria towards antibiotic resistance or poly-resistance would be inevitable as it represents a particular aspect of the general evolution of bacteria [17]. Although the antibiogram results in the present study show that *E. coli* is resistant to amoxicillin and penicillin with rates of 93.75% and 56.25% respectively. Resistance to ceftazidine and ceftriaxone, which are 3rd generation cephalosporins, was 43.75%. In addition, the rate of resistance to amoxicillin is higher than that reported by Hamez *et al.*, [18] who reported that 15% of *E. coli* strains were resistant to amoxicillin. Resistance to aminopenicillins (ampicillin) and first-generation cephalosporins in *E. coli* is often mediated by the production of β-lactamases that confer resistance to extended-spectrum cephalosporins or carbapenems. The spread of carbapenemase-producing Enterobacteriaceae, such as *E. coli*, is considered a major public health problem due to the inability to treat the infections they cause [19]. Klebsiella strains show resistance to amoxicillin (75%), ampicillin and penicillin (50%) through the production of a chromosomal penicillinase. Our results are lower than those obtained by Amin *et al*, (2017) [1] (88.9%) and Gomatheswari and Jeyamurugan, (2018) [8] (83%) for ampicillin. The 6 *Klebsiella pneumoniae* strains also showed resistance to 3rd generation antibiotics: Cefoxime and ceftaxidine (25%). The carbapenemase-producing strains in this study are less frequent than those reported by Sharma *et al*., in 2021 [6]. For *S. aureus*, several studies report that it is the most common cause of skin and soft tissue infections and that MRSA is a public health problem [20]. In this study, over 72% of the strains were resistant to penicillin G (penicillinase) and 23 strains (42.60%) were resistant to oxacillin (MRSA). They are therefore resistant to beta-lactams. These results are superior to those of a Libyan study, which reported low levels of resistance to ciprofloxacin (3.31%), penicillin (34.93%), erythromycin (18.02%) and gentamicin (2.94%) [20]. *Pseudomonas aeruginosa* is the species with the least resistance to the antibiotics tested. The highest rates of resistance are found for piperacillin and gentamicin (33.3%), ticarcillin (16.6%) and ciprofloxacin (50%) compared to those reported by Sami in 2012 [21] and Li *et al.*, in 2016 [22]. For aminoglycosides (gentamicin), the rate is 33.3%. This rate is higher than the rate reported in an Ethiopian study, which was 7.8% [20]. *P. aeruginosa* has several three-component efflux systems, some of which confer resistance to β-lactams when they are highly expressed.

6. Conclusion

Suppurative infections are common infections that can be caused by a variety of microorganisms and require antibiotic therapy. Excessive and sometimes inappropriate use of antibiotics has led to the emergence of antibiotic-resistant bacteria. It is therefore important to control the epidemiological profile of bacterial infections and their susceptibility to antibiotics. The results obtained in this study show that purulent samples have a positivity rate of 43.51%. The cytobacteriological examination of purulent samples allowed us to isolate 114 germs, with a predominance of gram-positive bacteria (53.5%). The most frequently isolated species were *Staphylococcus* *aureus* (47.37%), *Escherichia coli* (14.04%) and Klebsiella (7%). Analysis of the antibiogram results for the isolated strains revealed high levels of resistance to the main families of antibiotics tested, particularly in Enterobacteriaceae. *Klebsiella pneumoniae* showed high levels of resistance to beta-lactams, including third-generation cephalosporins, and to quinolones.

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