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Article

Microbiological Profile and Their Antibiotic Susceptibility Pattern—Experience in a Tertiary Care Hospital in Bangladesh

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Abstract: Background: It is essential to monitor causative agents of infections and antimicrobial resistance patterns to inform treatment and policy at the local level. In this study, we investigated the microbiological profile and antibiotic susceptibility pattern records in a tertiary care hospital. **Materials and Method:** This cross-sectional study was performed in a tertiary care hospital in Dhaka city, Bangladesh. The study utilized a retrospective descriptive research approach conducted between January 2018 and February 2021 in which culture results of blood, stool, urine, body fluid, genital, respiratory and soft tissue specimens were retrieved and analyzed. **Results:** A total of 26,825 samples were analyzed; of which 3,779 records for microbial growth from clinical specimens were identified, yielding a 14.09% isolation rate. Escherichia coli (E. coli), Klebsiella sp., Staphylococcus aureus, Pseudomonas sp., coagulase negative Staphylococcus, Salmonella Typhi and Enterococcus sp. were the most frequently isolated organisms among all specimens. E. coli, the most common causative organism of urinary tract infection (UTI) and genital infection, showed high resistance to co-trimoxazole (48%), ciprofloxacin (79%), and cephalosporins (63-65%) while the resistance rate to nitrofurantoin (7%), mecillinam (16%), aminoglycosides (7-18%), meropenem (8%) and colistin (0%) was low. In bloodstream infection, the most common microorganism found was S. Typhi. High sensitivity towards amoxicillin (100%), chloramphenicol (78%), co-trimoxazole (76%), cefixime (100%) and ceftriaxone (100%) were seen in S. typhi whereas almost all isolates were resistant to nalidixic acid (97%) and ciprofloxacin (96%). S. aureus, the predominant cause of soft tissue infection,

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was highly sensitive to co-trimoxazole (70%), doxycycline (86%), and linezolid (97%). Methicillin-resistant Staphylococcus aureus (MRSA) was 54%. The most common cause of respiratory infection was Klebsiella sp. The rate of resistance of Klebsiella sp. to third and fourth generation cephalosporins was approximately 65% and to ciprofloxacin and meropenem was 74% and 42% respectively. Pseudomonas sp., found commonly in urine, soft tissue, body fluids, and respiratory infections, were susceptible to ceftazidime, piperacillin/tazobactam and ciprofloxacin with resistance rate of 27%, 18% and 34% respectively. High resistance to meropenem was recorded for Pseudomonas sp. (30%) and Acinetobacter sp. (64%). Acinetobacter sp. showed low sensitivity (< 45%) to all of the tested antibiotics except colistin and tigecycline. **Conclusion:** This study highlights the high potential for infections associated with resistant microorganisms in the tertiary care hospital where samples for this study were collected. It is therefore recommended that judicious treatment following drug-susceptibility testing of isolates, regular surveillance programs, and strict infection control measures be put into place to address this increasing drug resistance problem.

Keywords: AMR Surveillance; antimicrobial susceptibility testing; antimicrobial resistance; QAAPT; WHONET

1. Introduction

Sir Alexander Fleming's ground-breaking discovery of penicillin in 1928 marked the beginning of the antibiotic revolution, which fundamentally altered the direction of contemporary medicine [1]. Antibiotics have successfully increased life span and are currently most often prescribed drugs in hospitals around the world [2]. However, the rising resistance to antibiotics is alarming. The high prevalence of infectious diseases, the absence of adequate guidelines for the therapy of infections and facilities for infection control [3], and the irrational prescribing, dispensing, and administration of antibiotics [4] primarily contribute towards the rapid development of antibiotic resistance worldwide. Moreover, due to the cumbersome diagnostic process, antimicrobial drugs are occasionally started empirically, which may facilitate the emergence of drug resistant strains of bacteria [5]. For instance, in Africa, treatment recommendations for infections rely heavily on the use of empiric antibiotics without the support of culture results [6]. Antibiotic resistance causes a rise in morbidity, mortality, hospital stay time, and medical costs [7]. Controlling AMR requires routine observation of the pathogenic organisms and their antibiotic susceptibility profile [8]. The AMR surveillance data will guide physicians for judicious selection of antibiotics, inform the policy resulting in improved patient care [9]. However, the majority of healthcare providers lack current antimicrobial resistance (AMR) data [10]. This data is needed to institutions and hospitals to formulate relevant policies and ensure best prescription practices [11].

The Capturing Data on Antimicrobial Resistance Patterns and Trends in Use in Regions of Asia (CAPTURA) initiative, funded by the Fleming Fund Regional Grants and led by the International Vaccine Institute (IVI), aimed to significantly increase the volume of available AMR (Antimicrobial Resistance), AMC (Antimicrobial Consumption), and AMU (Antimicrobial Use) data for informed decision-making as well as assess the quality of datasets and laboratories. By collaborating with local governments and both private and public healthcare facilities, the initiative identified and assessed data that were often unused, evaluating them for quality and availability. Relevant data were then shared with CAPTURA, where they were collated and analyzed to provide insights at local, regional, and interregional levels.

This study was conducted at Bangladesh Specialized Hospital (BSH), a tertiary level hospital in Dhaka, to evaluate the microbiological profile of various bacterial infections and their antimicrobial susceptibility patterns with the technical support from the CAPTURA project. The findings guide future initiatives in encouraging awareness, policy, and interventions to combat the urgent global threats of spreading AMR and antimicrobial misuse.

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2. Materials and Methods

Study design and place

A cross-sectional study was conducted in Bangladesh Specialized Hospital (BSH), a tertiary care hospital in Dhaka city, Bangladesh. The current study utilized a retrospective descriptive research approach where culture results of specimens of blood, genital, respiratory, soft tissue and body fluids, stool and urine at the Microbiology Department in BSH were retrieved were analysed for the period between January 2018 and February 2021 in which.

Data collection and processing

A total of 26,825 samples were included in the study. Data related to demographics (age and gender), type of specimens, type of microorganism, and antibiotic sensitivity/resistance pattern were retrieved from both paper and electronic data recording systemthe medical records. For bacterial isolation, identification and drugs susceptibility testing, samples were collected, processed, and cultured following standard techniques used in medical microbiology laboratory. For pathogen identification, colonies formed were further processed using morphology, Gram staining, and biochemical tests. Antibiotic susceptibility testing of bacterial isolates was performed using the Kirby Bauer disc diffusion method and observations were interpreted in accordance with guidelines set by the National Committee for Clinical Laboratory Standards (CLSI). Due to resource limitation, fastidious bacteria and any species requiring complex nutritional components and specialized detection methods were not included in the routine clinical laboratory diagnosis.

Statistical analysis

Descriptive statistics were applied to the collected data using WHONET and Quick Analysis of Antimicrobial Patterns and Trends (QAAPT) software. Results are expressed in frequency distributions and antibiograms.

3. Results

3.1. Demography and bacterial culture

Between January 2018 - February 2021, a total of 26,825 clinical specimens were processed for culture and identification. Bacterial growth was detected in 14% (n = 3779) of the samples. The patient demography showed approximately equal distribution of bacterial infections in both sexes (female and male, 52% and 48% respectively). In term of age distribution, the median age group for male was 45-54 years, while for female this remained 35-44 years (Figure 1).

The specimen distribution showed urine as the commonest specimen (48.7%) collected for bacterial culture followed by blood (27.1%) and soft tissue and body fluid (8.1%). The culture positivity rate for urine, blood and soft tissue and body fluid was 41%, 24% and 15% respectively (Table 1). All sample types of *Escherichia coli*, *Klebsiella sp.*, *Staphylococcus aureus*, *Pseudomonas sp.*, *coagulase negative Staphylococcus*, *Salmonella Typhi* and *Enterococcus sp.* were common pathogens (Figure 2). A detailed distribution of microorganisms in different specimens is presented in Figure 3. Overall, *E. coli* was predominant in urine and genital tract samples, *Salmonella Typhi* in blood, *Staphylococcus aureus* from soft tissue and body fluids, and *Klebsiella sp.* from the respiratory tract.

3.1.1. Antibiotic susceptibility pattern

A thorough overview of antibiotic sensitivity pattern of both Gram-positive and Gram-negative bacteria are depicted in Table 2 and Table 3. *E. coli*, the most common causative organism of Urinary Tract Infection (UTI) and genital infection, showed high resistance to co-trimoxazole (48%), ciprofloxacin (79%) and amoxicillin-clavulanic acid (65%) though nitrofurantoin and mecillinam were observed highly susceptible with resistant rate of only 7% and 16% respectively. Resistance of

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E. coli to third generation cephalosporin such as cefotaxime, ceftazidime and ceftriaxone was 63%, 64% and 63% respectively. However, resistance to amikacin and meropenem was 7% and 8% respectively in *E. coli*. In systemic infection, the principal microorganism found was *Salmonella Typhi*. High sensitivity towards amoxicillin (100%), chloramphenicol (78%), co-trimoxazole (76%), cefixime (100%) and ceftriaxone (100%) were seen in *Salmonella Typhi* whereas almost all isolates were resistant to nalidixic acid (97%) and ciprofloxacin (96%). *Staphylococcus aureus*, the predominant cause of soft tissue infection, was highly sensitive to recommended drugs for uncomplicated soft tissue infection such as co-trimoxazole (70%) and doxycycline (86%), and for complicated infection vancomycin (99%) and linezolid (97%). Methicillin-resistant Staphylococcus aureus (MRSA) was detected by testing susceptibility to cefoxitin, with 54% isolates found to be MRSA. The most common cause of respiratory infection was *Klebsiella sp*. *Klebsiella sp*. was the most commonly tested pathogen in patients with respiratory infectionThe rate of resistance of *Klebsiella pneumoniae* to third generation cephalosporin such as cefixime, cefotaxime, ceftazidime, ceftriaxone and fourth generation cephalosporin like cefepime was about 65%. Though high resistance of the bacteria to ciprofloxacin (74%) and meropenem (42%) was documented, colistin was 100% sensitive to *Klebsiella pneumoniae*. *Pseudomonas sp*., commonly tested in urine, soft tissue and body fluids, and respiratory infections, were susceptible to ceftazidime, piperacillin/tazobactam and ciprofloxacin with resistance rate of 27%, 18% and 34% respectively. High resistance to meropenem was recorded in *Pseudomonas sp*. isolates (26%) and *Acinetobacter sp.* (64%). *Acinetobacter sp.* showed low sensitivity (< 45%) to all of the tested antibiotics except colistin and tigecycline. *Enterococcus sp*., recorded commonly from urine sample, was sensitive to amoxicillin (72%) and nitrofurantoin (74%) while low sensitivity was exhibited to ceftriaxone (30%), amikacin (25%) and ciprofloxacin (15%). Moreover, 1% vancomycin-resistant Enterococcus (VRE) and 2% linezolid-resistant Enterococcus sp. were observed.

3.2. Figures, Tables and Schemes

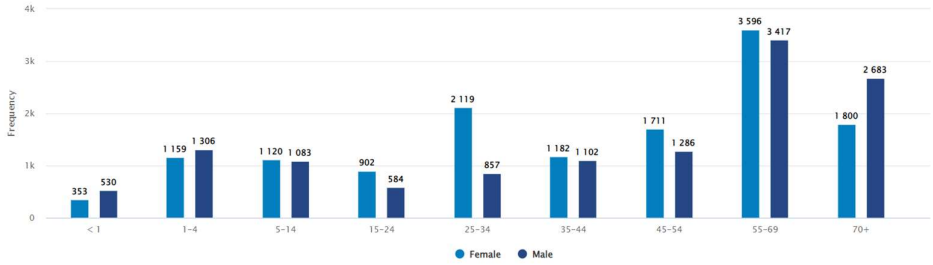

Figure 1. Distribution of isolates according to age and sex.

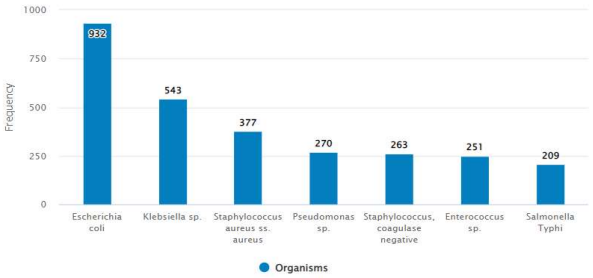

Figure 2. Percentage of organisms isolated from all samples over the reported.

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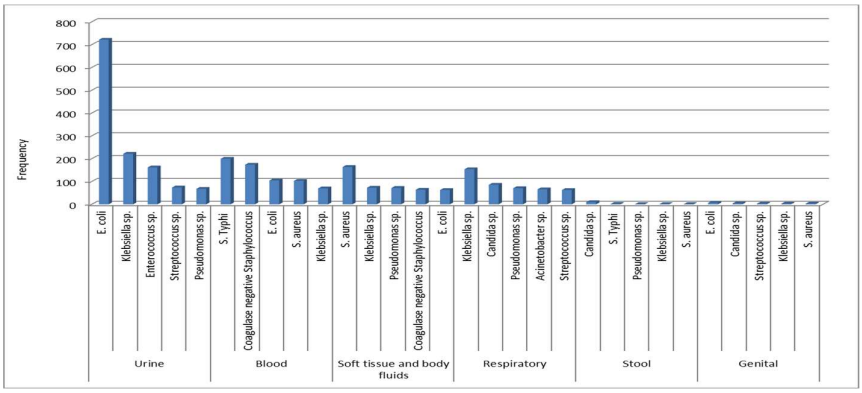

Figure 3. Most common organisms by specimen category. Urine, Blood, Soft tissue and body fluids (& BF), Respiratory, Stool, and Genital.

Table 1. The number of culture records stratified by specimen category.

Specimen	All Isolates N (%)	Positive culture n (%)	Negative culture n (%)
Urine	13059 (48.7)	1549 (41)	11510 (49.9)
Blood	7274 (27.1)	902 (23.9)	6372 (27.6)
Soft tissue and body fluids	2160 (8.1)	567 (15)	1593 (6.9)
Respiratory	2012 (7.5)	645 (17.1)	1367 (5.9)
Stool	1130 (4.2)	17 (0.4)	1113 (4.8)
Genital	218 (0.8)	23 (0.6)	195 (0.8)
Others	972 (3.6)	76 (2)	896 (3.9)
Total	26825	3779 (14.09)	23046 (85.91)

Table 2. Gram positive antibiogram. The numbers indicate % susceptible.

Organism	Number of patients*	AMC	AMK	AZM	CIP	CLI	CRO	CTX	CXM	DOX	FEP	FOX	GEN	LNZ	NET	NIT	PEN	SXT	TCY	TEC	VAN
Staphylococcus aureus	366	46	90	20	36	55			42	86	46	46	73	97	96		23	70		99	99
Staphylococcus epidermidis	260	39	91	16	46	61			38	92	39	39	68	96	97		32	63		100	100
***Enterococcus* sp.**	247	72	25	9	15	11	30	31		63	29		68	98	61	74	66	10	38	90	99
Streptococcus pyogenes	92	100	35	39	24	59	100	100		92	100		100	99	87	97	100	3		96	100
Streptococcus agalactiae	40	100		49	28		100	100		90	100			100		100	100	2			100

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Note: *Antibiogram table created using number of patients (n=1,005) rather than number of isolates. AMC-amoxicillin/clavulanic acid, AMK-amikacin, AZM-azithromycin, CIP-ciprofloxacin, CLI-clindamycin, CRO-ceftriaxone, CTX-cefotaxime, CXM-cefuroxime, DOX-doxycycline, FEP-cefepime, FOX-cefoxitin, GEN-gentamicin, LNZ-linezolid, NET-netilmicin, NIT-nitrofurantoin, PEN-penicillin G, SXT-co-trimoxazole, TCY-tetracycline, TEC-teicoplanin, VAN-vancomycin.

Table 3. Gram negative antibiogram. The numbers indicate % susceptible.

Organism	Number of patients*	AMC	AMK	ATM	AZM	CAZ	CFM	CHL	CIP	COL	CRB	CRO	CTX	FEP	GEN	MEC	MEM	NAL	NET	NIT	PEF	SXT	TCY	TGC	TOB	TZP
Escherichia coli	922	35	93	35		36	35		21	100		37	37	37	82	86	92		92	93		52		87	70	78
Klebsiella pneumoniae	489	36	59	38		36	35		26	100		37	36	38	52	62	58		57	29		44	71	35	48	47
Pseudomonas aeruginosa	223	4	73	65		74			65	99	34			75	72		70		77					8	68	81
Salmonella Typhi	209	100	100	100	96	100	100	78	3	100		100	100	100	100		100	3	100		2	76			100	100
Acinetobacter sp.	181	4	34	5		21	4		40	94		4	3	19	32		36		42			69		92	33	44
Enterobacter sp.	139	53	91	64		66	51		66	90		66	65	69	85	80	96		90	51		77		55	82	91
Proteus sp.	53	74	82	79		79	74		28	2		76	76	83	67		98		85			32		50	75	94
Citrobacter freundii	47	43	94	42		44	44		37	98		43	43	45	83	74	87		92	82		50		91	72	83
Pseudomonas sp.	35		51	41		62			72	46				54	46		74		46					92	41	88
Salmonella Paratyphi	21	100					100			100		100	100	100			100					100				100

Note: *Antibiogram table created using number of patients (n=2,319) rather than number of isolates. AMC-amoxicillin/clavulanic acid, AMK-amikacin, ATM-aztreonam, AZM-azithromycin, CAZ-ceftazidime, CFM-cefixime, CHL-chloramphenicol, CIP-ciprofloxacin, COL-colistin, CRB-carbenicillin, CRO-ceftriaxone, CTX-cefotaxime, FEP-cefepime, GEN-gentamicin, MEC-mecillinam, MEM-meropenem, NAL-nalidixic acid, NET-netilmicin, NIT-nitrofurantoin, PEF-pefloxacin, SXT-co-trimoxazole, TCY-tetracycline, TGC-tigecycline, TOB-tobramycin, TZP-piperacillin/tazobactam.

4. Discussion

The overuse of antibiotics is highly associated with increased infections and costs, drug interactions, longer hospital stays, and bacterial resistance [12]. For successful use of empirical therapy and to prevent the emergence of antibiotic resistance, frequent investigation of the local epidemiology and the often-underestimated microorganisms' antimicrobial susceptibility pattern is necessary in developing countries [10].

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In the current analysis, 3,779 microbial growths were recovered from 26,825 samples yielding a 14.09% isolation rate. The higher proportion of female patients might be due to an enormous proportion of laboratory samples often coming from UTI in women. However, when analysing the distribution of the culture records by age group, a proportionally large number of records were in the higher age group for both males and females than might be anticipated. In the context of males, and particularly if the samples are urine samples, this observation is normal as males are more prone to urinary tract infections at later stages of life. Urine was the most common and frequently testedsample for culture. The culture positivity rate of blood cultures was low. This is because the isolation rate depends on various factors including the clinical condition, ambulatory or hospitalized state, and length of hospital stay and age of patient. *E. coli* was the topmost isolated organism. This correlates well with the fact that urine was the most common sample, as *E. coli* was often the most frequently reported pathogen in urine cultures. *Klebsiella sp., Pseudomonas sp.* and *Enterococcus sp.* were frequently isolated organisms in this setting. This could be the case if the facility is providing health care to more seriously ill patients requiring longer hospitalizations and higher amounts of antimicrobials. The number of isolates of *Salmonella Typhi* was also high. This finding is consistent with the fact that typhoid is endemic in Bangladesh and a large number of cases are reported every year with frequent outbreaks [13].

In this study, *E. coli*, a common cause of genitourinary tract infection, revealed high level of resistance to co-trimoxazole, ciprofloxacin and amoxicillin-clavulanic acid, while nitrofurantoin and mecillinam showed susceptibility to *E. coli*. A similar situation has been observed in developing countries [14]. About two-thirds of *E. coli* isolates showed resistance to third generation cephalosporin. South India reported 92% third generation cephalosporin-resistant *E. coli* in 2010 [3]. However, only 3.3% of ceftriaxone-resistant *E. coli* isolates were found in Ethiopia [15]. Resistance to amikacin and meropenem was 7% and 8% respectively in *E. coli*. In south India, 12% amikacin-resistant and 16% meropenem-resistant *E. coli* were reported [3].

In systemic infections, the most common causative agent was *Salmonella Typhi*, which showed high sensitivity towards amoxicillin (100%), chloramphenicol (78%), co-trimoxazole (76%), cefixime (100%) and ceftriaxone (100%) were seen in *Salmonella Typhi* whereas almost all isolates were resistant to nalidixic acid and ciprofloxacin. The gradual upward trend in sensitivity to co-trimoxazole, chloramphenicol and amoxicillin and downward trend in sensitivity to ciprofloxacin and nalidixic acid have been observed through recent years [13,16]. *Staphylococcus aureus*, causing soft tissue infection predominantly, was highly sensitive to recommended drugs for uncomplicated soft tissue infection such as co-trimoxazole (70%) and doxycycline (86%) and for complicated infection vancomycin (99%) and linezolid (97%). Global surveillance indicates > 90% susceptibility of *Staphylococcus aureus* to co-trimoxazole, providing high confidence for empiric use in uncomplicated skin and soft tissue infection [17]. In a previous study, doxycycline was reported to have 90% sensitivity against MRSA for uncomplicated skin and soft tissue infection [18]. In this study, 54% of isolated *Staphylococcus aureus* were methicillin resistant. These results is consistent with other studies [15,16]. Of the staphylococcal groups, MRSA is associated with a greater risk of mortality, longer duration of hospitalization and higher hospital costs compared with methicillin-susceptible *Staphylococcus aureus* [19-21]. Vancomycin is the drug of choice for MRSA and linezolid is the drug of choice for vancomycin-resistant *Staphylococcus aureus* (VRSA). However, alarmingly 1% and 3% of isolates of *Staphylococcus aureus* were found resistant to vancomycin and linezolid respectively. High resistance to meropenem was recorded in *Pseudomonas sp.* isolates (26%) and *Acinetobacter sp.* (64%). This observation is in agreement with the study reported by Ahmed et al[16]. In south India, the resistance of *Acinetobacter sp*. to meropenem increased from 16% in 2007 to 70% in 2008 [3]. In this study, 1% vancomycin-resistant *Enterococcus* (VRE) was observed. No isolates of VRE were reported in previous studies in Bangladesh [16]. However, VRE is prevalent in many countries [22-24]. More extensive research needs to be done in Bangladesh to obtain a definitive insight.

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5. Conclusions

This study revealed that *E. coli, Klebsiella sp., S. aureus, Pseudomonas sp., coagulase negative Staphylococcus, S. Typhi and Enterococcus sp*. were the most common isolates in clinical samples. The prevalence of antimicrobial resistance is very high. Moreover, third generation cephalosporin-resistant Enterobacteriaceae, carbapenem resistant *Acinetobacter sp., P. aeruginosa* and *E. coli*, MRSA, VRSA, linezolid-resistant *Staphylococcus aureus*, VRE and ciprofloxacin-resistant *Salmonella Typhi* are public health alerts. Policy makers and national stakeholders should prioritize actions such as surveillance programs, audits, the creation of national antimicrobial guidelines, strong infection control procedures to control the threat of AMR. In addition, there is also a strong need for periodical training and education for healthcare professionals to abate the incidence of AMR in the country.

Author Contributions: MJS reports to lead the data collection, management, and analysis. MZA, FR, SF, SMSR, AR, ZHH, AS, HTB, SG, FM, and NP edited the primary draft. All authors read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The CAPTURA project was exempt from ethical review by the Institutional Review Board (IRB) of the IVI because the project did not involve intervention or interaction with individuals and the information collected was not individually identifiable. This exemption is per the IVI IRB SOP D-RB-4-003. The CAPTURA project undertook the retrospective data collection and curation, and the authors used the digitized data to prepare this manuscript.

Informed Consent Statement: The CAPTURA (Capturing data on Antimicrobial Resistance Patterns and Trends in Use in Regions of Asia) consortium project received an official approval from the Communicable Disease Control, DGHS (Directorate General of Health Services), MoHFW (Ministry of Health and Family Welfare) dated on May 17, 2020. The reference number is DGHS/DC/ARC/2020/1708. Prior to the data collection, a tri-party collaborative agreement was made among the DGHS, Bangladesh Specialized Hospital, and International Vaccine Institute- CAPTURA on 14 October 2020.

Data Availability Statement: The dataset will be shared upon request.

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Conflicts of Interest: Declare conflicts of interest or state "The authors declare no conflicts of interest."

Abbreviations

The following abbreviations are used in this manuscript:

BSH	Bangladesh Specialized Hospital
CAPTURA	Capturing Data on Antimicrobial Resistance Patterns and Trends in Use in Regions of Asia
IEDCR	Institute of Epidemiology Disease Control & Research
CDC	Communicable Disease Control
MoHFW	Ministry of Health and Family Welfare
QAAPT	Quick Analysis of Antimicrobial Patterns and Trends
CLSI	Clinical & Laboratory Standards Institute
VRSA	Vancomycin-resistant *Staphylococcus aureus*
MRSA	Methicillin-resistant Staphylococcus aureus

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