Magnitude, Diversity and Prevalence of Antibiotic Resistant Bacteria in Pediatric Patients of Coastal Area at Khulna Region, Bangladesh

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ABSTRACT

Antibiotic resistance is one of the biggest public health challenges around the world. A cross-sectional study was conducted to elucidate the magnitude, diversity and antibiotic resistant pattern of coastal pediatric patients. All isolates were characterized by routine bacterial culture, gram staining and biochemical tests. A total of 120 samples from various body fluids of pediatric patients were taken from 3 hospitals in Khulna city. Data were analysed by Excel 2016 and Graphpad Prism (8.0.2). 81 samples (67.5%) showed positive growth, among which 45 (75%) and 36 (60%) were coastal and non-coastal patient's sample respectively. Out of 9 identified bacteria, 6 were found in both coastal and non-coastal pediatric patients namely, *E.coli*, *Staphylococcus aureus*, *Klebsiella spp.*, *Streptococcus spp.*, *Salmonella spp.*, *Pseudomonas spp.* whereas, *Corynebacterium spp.* found only in coastal and *Acinetobacter spp.*, & *Shigella spp.* were found in only non-coastal pediatric patients. Gram negative bacteria were most prevailed both in coastal (66.7%) and non-coastal (72.2%) patients. Moreover, Urinary Tract Infections (UTIs) were more prevalent at early childhood stage (2-5 years of age) in coastal area whereas, infants (28 days-12 months of age) were more prone to pneumonia in non-coastal area. Antibiotic resistance patterns for 10 commonly prescribed antibiotics through Kirby-Bauer disk diffusion method reveals, most of the bacterial strains showed multidrug resistant (≥ 80%) against 7 and/or more classes of antibiotics. Therefore, it is necessary to reduce the abundant use of antibiotics. Institutional antimicrobial resistance surveillance efforts and use of biogenic nanomaterials and microalgae-based technologies could decrease the occurrence.

Keywords: Coastal, Pediatric patients, Antibiotic resistance, Urinary Tract Infections, Surveillance.

INTRODUCTION

Antibiotic resistance poses a significant public health challenge in countries worldwide (Kraemer et al., 2019). The high prevalence of antibiotic-resistant bacteria in coastal regions is linked to fish farms and the presence of contaminated mobile ribosomal mutation as well as horizontal gene transfer (Labella et al., 2013). The World Health Organization (WHO) has recognized antibiotic resistance as one of the top ten global public health threats and estimated that bacterial antimicrobial resistance (AMR) was directly responsible for 1.27 million deaths worldwide in 2019, a number that could rise to 10 million annually by 2050 (*Antimicrobial Resistance*, 2023). There are several notable risk factors linked to the presence of antimicrobial-resistant (AMR) bacteria in the pediatric population of the Asia-Pacific region (Chan et al., 2022). The coastal zone of Bangladesh is recognized for its significant potential and heavy human activity, making it highly susceptible to both natural and human-made hazards (Parvin et al., 2015). Around 35 million people (29%) of this country live in Coastal area including Khulna 2.6 million (Ahmad, 2019). The main livelihood of this area include fishing, salt-shrimp processing, fish drying, agriculture, tourism, and small-scale trading business (Hossain et al., 2017).

Antibiotic resistance is a growing public health concern, particularly significant for children (Romandini et al., 2021). The wetland habitat raises concern regarding the potential transmission of antibiotic resistance genes to native bacteria as a result of human activities, even in high-salinity environments (Bergeron et al., 2016). Salinity is a crucial factor in influencing the distribution patterns of antibiotic resistance genes in both oceans and rivers (Zhang et al., 2019). Shrimp farming could contribute to the global spread of antimicrobial resistance (AMR) (Thornber et al., 2020).

Coastal areas around the world have reported a high prevalence of antibiotic resistance genes (ARGs), particularly near urban and hospital sewage disposal sites (Guillén-Chable et al., 2024) antimicrobial resistance genes (ARGs) have been detected in these coastal regions (Campanini-Salinas et al., 2024) as well as in coastal water. One disposal option for antibiotics is through trash or drains, which can lead to their accumulation in the environment. This accumulation may promote the development of antimicrobial resistance mechanisms and facilitate their transfer between microorganisms (Campanini-Salinas et al., 2024).

Floodwaters can pollute soils and irrigation systems, affecting crop and livestock production. This contamination can introduce antimicrobial-resistant microorganisms into the food chain, further exacerbating the issue of antimicrobial resistance (Furlan et al., 2024). Evidence indicates that increasing salinity levels are associated with various health issues, including skin diseases, acute respiratory infections, cardiovascular diseases, strokes, and diarrheal diseases (Rahman & Ahmad, 2018). Moreover, the severity of infections, including antibiotic-resistant infections, is rising as the effects of climate change intensify and accelerate (Burnham, 2021).

Escherichia coli is the most common pathogen associated with urinary tract infections (UTIs), responsible for 85% to 90% of cases (Barola et al., 2024). *Staphylococcus aureus* is a significant cause of pneumonia-related hospitalizations among young children worldwide (Kulkarni et al., 2022). *Klebsiella pneumoniae* is a major concern for pneumonia in children, and antibiotic-resistant *Klebsiella spp.* pose an increasing public health risk (Verani et al., 2024). Klebsiella species can lead to severe infections, including pneumonia, urinary tract infections, bloodstream infections, and meningitis (*Antimicrobial Resistance, Hypervirulent Klebsiella Pneumoniae - Global Situation*, n.d.). The most vulnerable groups include children under 12 months and adults over 70 years old. Additionally, men are more likely than women to develop resistant infections (*Five Reasons to Care about Antimicrobial Resistance (AMR)*, n.d.).

Specific pediatric challenges, including inappropriate antibiotic prescriptions for incorrect diagnoses, limited treatment options, a lack of clinical trials involving children, and the changing dynamics of this diverse population, contribute to this complex situation (Romandini et al., 2021).

Wastewater treatment plants (Singh et al., 2022), Ultrafiltration, ultraviolet (UV) irradiation, anaerobic membrane bioreactors are several advanced techniques employed for water treatment and purification (Mosaka et al., 2023) methods are employed for the inactivation of antibiotic-resistant bacteria and the removal of antibiotic-resistant genes. There should be a more judicious use of antibiotics in the shrimp farming process (Palacios et al., 2021). Biogenic nanomaterials and microalgae-based technologies are utilized to eliminate antibiotic-resistant bacteria and resistance genes in drinking water (Duarte et al., 2022). Moreover, facilitate and enhance clinical reasoning to improve the prescribing environment (Bassetti et al., 2022) , ensuring clean water, proper sanitation, the responsible use of antibiotics, and the appropriate disposal of unused antibiotics are essential measures (Bergeron et al., 2016) can help reduce the circulation of resistant bacteria in homes and communities (Maillard et al., 2020). So, this research characterize multi-drug resistant bacterial strains on coastal pediatric patients. Therefore, the aims of this study are:

i. Isolation & characterization of bacteria from body fluids like blood, urine, intestinal fluids, nasal mucus of pediatric patients
ii. Antibiotic resistant pattern of characterized bacteria against 10 commonly used antibiotics
iii. Elucidate the magnitude & diversity of contamination in coastal area

MATERIALS AND METHODS

Study Area and Sample Collection

This study was conducted on the patients of Pediatric Ward of 3 renowned hospitals (Ad-din Akij Medical College, Khulna Shishu Hospital and Khulna Medical College Hospital) in Khulna city from 16 June, 2023 to 10 January 2024. A total 120 samples from different body fluids like blood, intestinal fluids, urine, and nasal mucus were taken from the pediatric patients. Patient information i.e. age, gender, diseases and locality were filled in a questionnaire. Samples collection, transportation and processing were followed as per as WHO guidelines. Further research took place in the Animal Biotechnology Laboratory under the Biotechnology and Genetic Engineering Discipline at Khulna University, Bangladesh.

Sampling and Isolation of Isolates

All specimen collections were performed with the assistance of nurses. Body fluids like were collected from pediatric patient (upto 5 years old) maintaining aseptic condition. Patients who had been admitted for at least 1 or 2 days were considered in the study. Blood samples were collected in EDTA tubes, sterile vials were used to collect urine, autoclaved cotton swabs were used to collect nasal swabs and intestinal fluids from OG tube. Then, each sample was brought in a Zip-lock bag by labeling patient's name, age and sex. After that, samples were transported to the laboratory immediately using an ice box for further processing. Nutrient agar, blood agar, chocolate agar, and MacConkey agar media were prepared according to the manufacturer's instructions and sterilized by autoclaving (ALP CL-322) at a pressure of 15 psi at 121^{0}C for 15 minutes. Streak plate was done by inoculating loop at Laminar air flow (PSM ESCO AIRSTREAM 90 CM) maintained the aseptic condition. After plating in different agar media, Petri dishes were sealed by parafilm properly and then incubated (Incubator: BMT Venticell III Eco oven) at 37°C for 24 hours (Makwana et al., 2023).

Identification of Isolates

i) Bacterial routine culture: After incubation, colonies with specific characteristics like size, color, pigmentation, surface texture, elevation, edge were observed and confirmed the primary assumption of organisms (Giuliano et al., 2019).

ii) Gram staining: Bacterial strains were identified based on Gram's reaction. Prepared slide was examined under an electron microscope (Biobase bme-500d) to differentiate gram positive and gram negative bacteria based on size, shape and color (Tripathi & Sapra, 2024).

iii) Biochemical Tests: A total of 7 biochemical tests were done to identify the isolates which were Catalase Test, Oxidase Test, Methyl Red, Citrate Utilization Test, Triple Sugar Iron Test, Urease Test and Coagulase Test. Here mentioning that, Catalase and Coagulase tests were done to identify gram-positive isolates. Non-lactose gram negative bacteria were estimated by Triple Sugar Iron Test, Urease Test, Catalase Test and Oxidase Test. Besides, isolates of Enterobacteriaceae family and others were identified by series of tests like Catalase Test, Methyl Red, Citrate Utilization Test, Triple Sugar Iron Test, Urease Test. Finally, macroscopic characteristics, microscopic analysis and biochemical tests of isolated microorganisms were evaluated by standard laboratory method (Moore, 2021).

iv) Antimicrobial-Susceptibility Testing

The antimicrobial susceptibility profiles were completed by Kirby-Bauer disk diffusion method. An inoculum was prepared from an overnight pure culture in sterile normal saline solution (0.85%) in test tubes, maintaining the turbidity compare to 0.5 McFarland standard (*McFarland Standard Scale Value*, n.d.). Mueller-Hinton Agar plates were readied and streaking the pure colony contain suspension, used by sterile swabs and left for 3 minutes before the introduction of the antibiotic discs. The antibiotic sensitivity profiles of all isolates were analyzed using 10 different antibiotics (HiMedia), generally prescribed by the Physician. Selected antibiotics were i. Ciprofloxacin (5µg) ii. Amikacin, (30µg) iii. Imipenem (10µg), iv. Gentamicin (10µg), v. Cefixime (5µg), vi. Erythromycin (15µg), vii. Azithromycin (50µg), viii. Cefotaxime (30µg), ix. Colistin (10µg), and x.

Penicillin (10µg). The inhibition zones were analysed and set by National Committee for Clinical Laboratory Standards. The antibiotics used in the disc diffusion method, along with the diameter of their inhibition zones (in mm), were assessed according to the 2022 CLSI guidelines (*CLSI & Antimicrobial Susceptibility Testing (AST)*, n.d.).

Statistical Analysis

The data was analyzed using Excel 2016 (Microsoft Corporation) and GraphPad Prism 8.0.2 (GraphPad Software, Inc.)

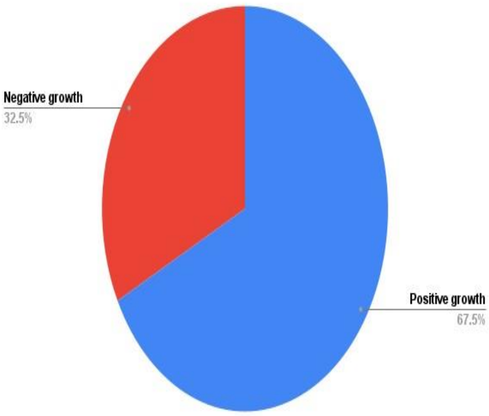
Ethical Considerations

The research received ethical approval and clearance from the Ethical Committee of Khulna Medical College Hospital, Record File No. 2606, Date: 15/06/2023. All the participants were briefed about the study and consent obtained from each of the participant. The information for each participant was obtained from hospital records using a numbered questionnaire. The serial number assigned to each questionnaire was preserved during the coding process, ensuring the participants' details remained anonymous. Written informed consent was obtained from each respondent, as well as from caregivers on behalf of children, before they were asked to provide data and samples. All information gathered during the study was kept confidential and used solely for research purposes.

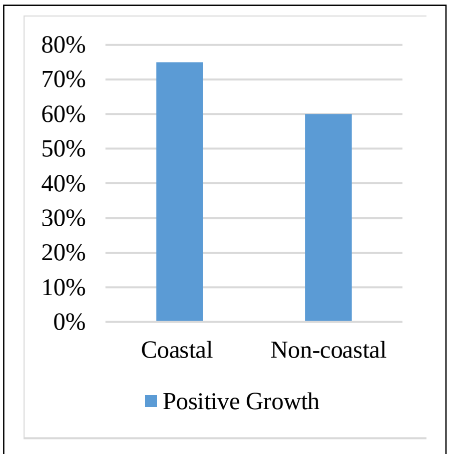
RESULTS

Bacterial Growth

A total of 120 samples from body fluids of pediatric patients were taken from 3 different hospitals in Khulna city. The samples were categorized on the basis of patient's locality (coastal and non-coastal area). Of them, 60 samples were from Coastal pediatric patients and rest 60 were from Non-coastal pediatric patients. Among the samples, 81 (67.5%) showed positive growth (figure 1) in where coastal patient's samples showed 45 (75%) and non-coastal patient's sample showed 36 (60%). However, 39 samples showed negative growth which were discarded.



(a)



(b)

Figure 1: (a) Growth percentage of 120 samples and (b) Positive growth of isolates from coastal and non-coastal patient's samples

This study revealed that, samples from coastal area's (Koyra, Paikgacha, Dakop, Batiaghata) showed more positive growth than samples from non-coastal area's (Dumuria, Rupsha, Digholia, Terokhada, Phultola) in Khulna district (figure 2).

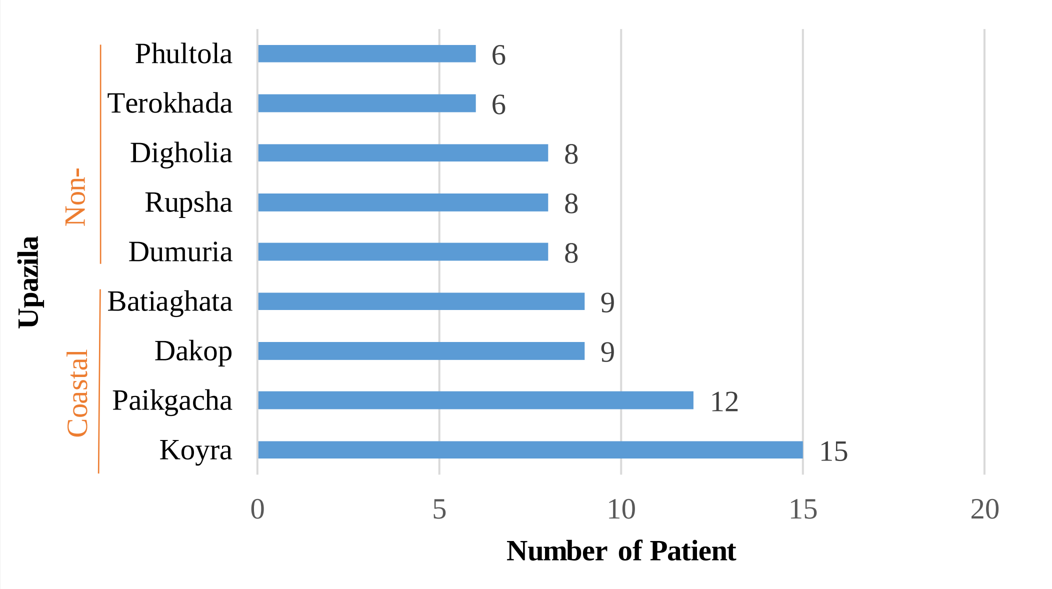


Figure 2: Positive growth samples from body fluids of pediatric patients at 9 Upazilas in Khulna District

Body fluids such as blood samples showed higher (56%) whereas, urine samples showed lower (4%) positive growth (figure 3).

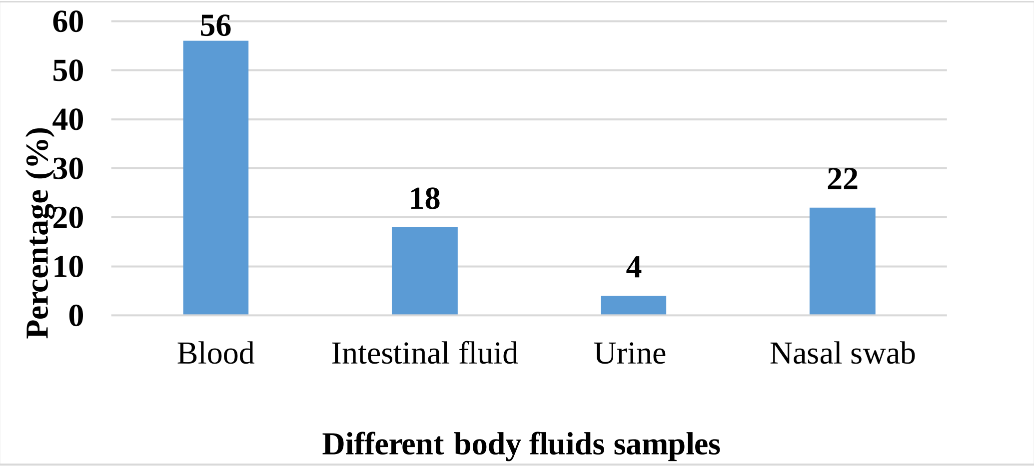


Figure 3: Growth percentage of isolates from different body fluids samples

Identification of Isolates

i) Colony Morphology Colony of the isolates was characterized based on elevation, shape, size, color etc. by growing on different agar media are presented in table 1.

Table 1: Macroscopic Observation of pure culture

No. of Isolates	Elevation	Shape	Nutrient Agar	Blood Agar	Chocolate Agar	MacConkey Agar
21	Convex	Round	Creamy white	Grey white	Grey white	Pinkish red
20	Convex	Irregular	Golden yellow	Beta hemolysis	Beta hemolysis	No Growth
5	Convex	Irregular	Translucent	Beta hemolysis	Colorless	Colorless

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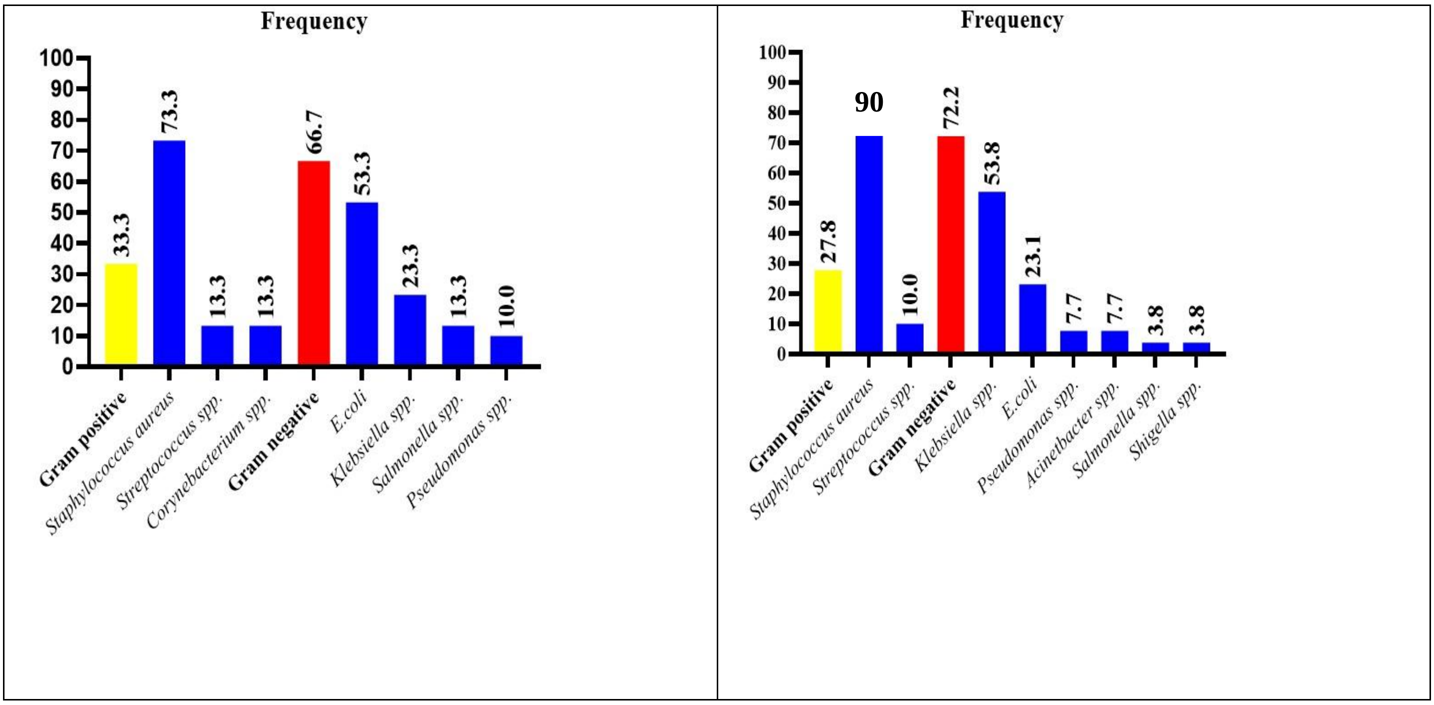
5	Convex	Round	Transparent	Grey white	Grey white	Colorless
22	Convex	Irregular	Translucent	Gray and moist	Gray and moist	Pink
2	Flat	Round	Gray	Narrow beta hemolysis	Narrow beta hemolysis	No Growth
2	Convex	Round	Gray	Non-pigmented	Non-pigmented	Light pink
3	Convex	Round	Sparkling colony	Greenish halo	Greenish yellow	No Growth
1	Convex	Round	Colorless	Gray and non-pigmented	Greenish	Slightly pink

ii) Gram Staining

Gram positive isolates were 33.3% in coastal and 27.8% in non-coastal pediatric patients whereas, the remaining isolates were gram negative (Figure 4)



(a) Coastal (b) Non-coastal

Figure 4: The proportion of isolated bacteria (gram positive & negative) from (a) coastal and (b) non-coastal pediatric patients

iii) Biochemical Test Results Different biochemical tests were done (table 2 & table 3) for identification of isolates followed by gram staining.

Table 2: Biochemical test results of the isolates

Test	**+ve (Positive)**	**-ve (Negative)**	**% Positive**
Oxidase	5	76	6.17%
Catalase	30	51	37.04%

Citrate	33	48	40.74%
Methyl Red	47	34	58.02%
Urease	41	40	50.62%
Coagulase	20	61	24.69%

Table 3: TSI test results of the isolates

Reaction	**No. of Isolates**	**Gas Production**	**Genus Identified**
Red/Yellow (+ Gas production)	20	Positive	**Staphylococcus spp.**
Red/Yellow (No gas)	4	Negative	**Shigella spp., Streptococcus spp.**
Red/Red (No gas)	5	Negative	**Pseudomonas spp.**
Red/Red (+ Gas production)	2	Positive	**Acinetobacter spp.**
Yellow/Yellow	43	Positive	**Escherichia spp., Klebsiella spp.**
Red/Black (+ Gas production)	7	Positive	**Salmonella spp., Corynebacterium spp.**

iv) Estimated Bacterial Strain The biochemical test results estimated 9 different genus of bacteria (table 4) found namely, *E.coli*, *Staphylococcus aureus, Klebsiella spp.*, *Corynebacterium spp.*, *Streptococcus spp.*, *Salmonella spp.*, *Pseudomonas spp.*, *Acinetobacter spp. and Shigella spp.*

Table 4: Estimated bacterial strains from various biochemical tests

Estimated bacteria	
1	*E.coli*
2	*Staphylococcus aureus*
3	*Klebsiella spp.*
4	*Streptococcus spp.*
5	*Pseudomonas spp.*
6	*Salmonella spp.*
7	*Corynebacterium spp.*
8	*Shigella spp.*
9	*Acinetobacter spp.*

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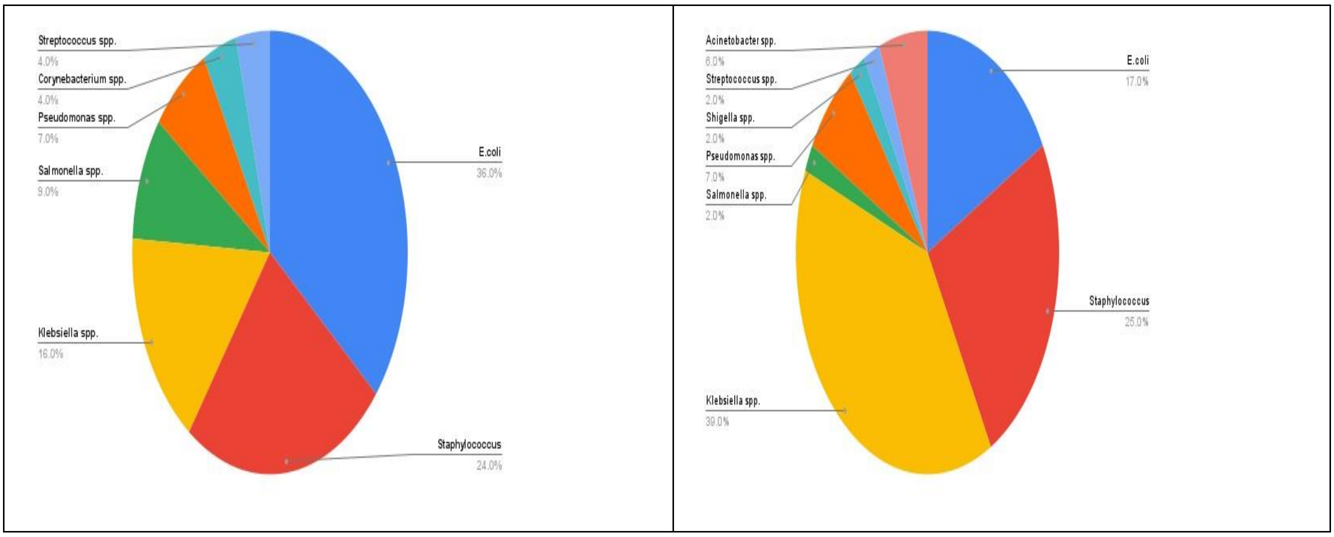
Among a total of 81 isolates, it showed (figure 4), the highest predominant gram negative bacteria (*E.coli*, *Klebsiella spp., Salmonella spp., Pseudomonas spp., Acinetobacter spp. and Shigella spp.)* both in coastal 30 (66.7%) and non-coastal 26 (72.2%) where, *E.coli* was highest in coastal and *Klebsiella spp.* in non-coastal pediatric patients. In contrast, gram positive bacteria (*Staphylococcus aureus, Corynebacterium spp., Streptococcus spp.)* were present in coastal 15 (33.3%) and 10 (27.8%) in non-coastal patients where *Staphylococcus aureus* were highly observed from both patients.

Prevalence of Antibiotic Resistant Bacteria

The figure (Fig. 5) depict that, *E.coli* (36%) was the highest found predominant bacteria in coastal pediatric patients and *Klebsiella spp.* (39%) was the highest in non-coastal pediatric patients. 6 bacteria namely, *E.coli, Staphylococcus aureus, Klebsiella spp., Streptococcus spp., Salmonella spp., Pseudomonas spp.* were common to both coastal and non-coastal pediatric patients whereas, only *Corynebacterium spp.* in coastal pediatric patients and *Acinetobacter spp., Shigella spp.* in non-coastal pediatric patients were observed.

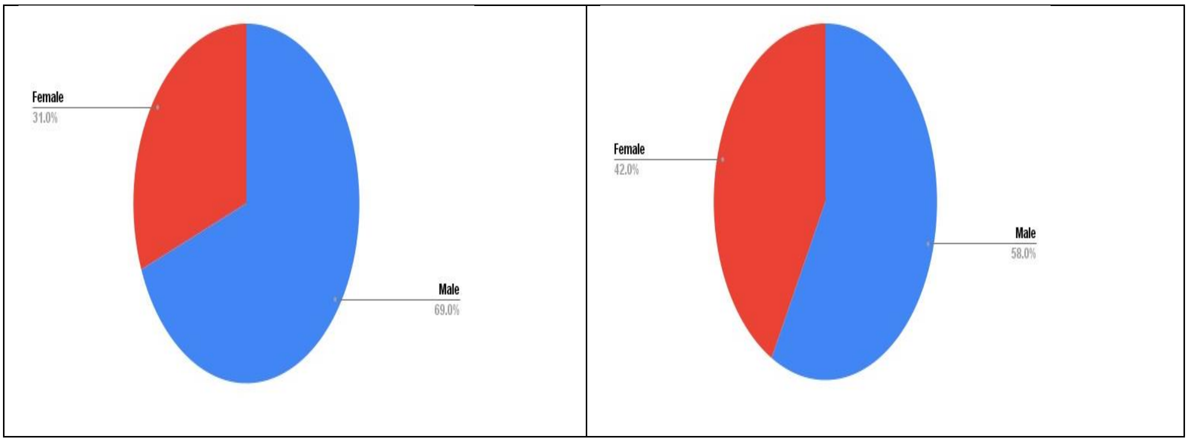


(a) Coastal (b) Non-coastal

Figure 5: Prevalence of different bacteria in (a) coastal & (b) non-coastal pediatric patients

Male-Female Infection

Coastal pediatric patients showed (figure 6), 69% infection on Male whereas, 31% infection on Female. In non-coastal pediatric patients, it showed 58% infection on Male whereas, 42% infection on female. So, both in cases, male patients were prone to infectious disease.



(a) Coastal (b) Non-coastal

Figure 6: Gender wise infection percentage in (a) coastal and (b) non-coastal pediatric patients

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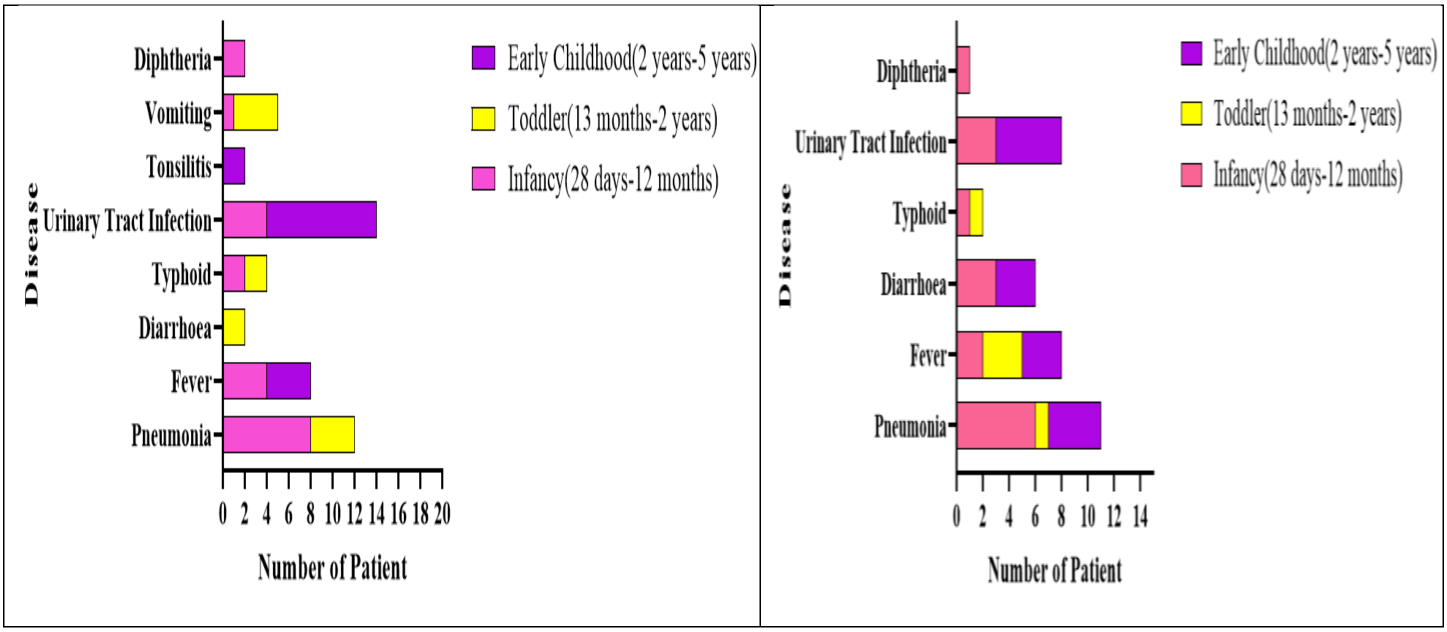
Infection Rate at Different Stages of Age

Pediatric patients from coastal area were mostly infected by Urinary Tract Infections (UTIs) and patients at early childhood (2 years-5 years) were mostly dominant (figure 7a). Besides, vomiting and tonsillitis were common in early childhood and toddler stages. Non-coastal pediatric patients were mostly infected by Pneumonia (figure 7b) at infancy stages (28 days-12 months).



(a) Coastal (b) Non-coastal

Figure 7: Disease infection rate at different ages in (a) coastal and (b) non-coastal pediatric patients

Antibiotic Susceptibility Testing

Kirby Bauer disk diffusion method revealed (table 5) that, For 100% resistant, *Staphylococcus aureus* against Penicillin and Ciprofloxacin, *Klebsiella spp.* against Cefixime and Colistin, *Corynebacterium spp.* against Ciprofloxacin, Imipenem, Gentamicin, Cefixime, Erythromycin, Azithromycin, Cefotaxime and Penicillin. *Streptococcus spp.* against Amikacin, Azithromicin, Cefixime, Erythromicin, Ciprofloxacin, Colistin and Penicillin, *Salmonella spp.* against Azithromicin, Cefixime, Ciprofloxacin and Colistin, *Pseudomonas spp.* against Ciprofloxacin, Colistin and Cefotaxime. Whereas, 100% sensitive *Corynebacterium spp.* to Amikacin and Colistin, *Streptococcus spp.* to Imipenem, Gentamicin and Cefotaxime, *Salmonella spp.* to Amikacin and Cefotaxime. Besides, 80%≥ resistant, *Staphylococcus aureus* against Cefixime (91%), Erythromicin (91%) and Colistin (81.8%), *E.coli* against Imipenem (81.3%) and Ciprofloxacin (81.3%), *Klebsiella spp.* against Cefotaxime(85.7%), Ciprofloxacin (85.7%) and Penicillin (85.7%).

Table 5: Antimicrobial-susceptibility patterns of bacteria isolated from different body fluids of Coastal patients

Bacterial Spp.	P	AK	AZM	CFM	IMP	GM	CTX	E	CIP	CT	P
Staphylococcus aureus (n=11)	S	4 (36.4%)	5 (45.5%)	1 (9%)	3 (27.3%)	7 (63.6%)	7 (63.6%)	1 (9%)	0	2 (18.2%)	0
	I	1 (9%)	1 (9%)	0	0	0	0	0	0	0	0

	R	6 (54.5%)	5 (45.5%)	10 (91%)	8 (72.7%)	4 (36.4%)	4 (36.4%)	10 (91%)	11 (100%)	9 (81.8%)	11 (100%)
E.coli (n=16)	S	5 (31.2%)	2 (12.5%)	3 (18.7 %)	3 (18.7%)	5 (31.2%)	3 (18.7%)	6 (37.5%)	3 (18.7%)	3 (18.7%)	3 (18.7%)
	I	1 (6.2%)	4 (25%)	2 (12.5 %)	0	2 (12.5%)	1 (6.2%)	1 (6.2%)	0	2 (12.5%)	1 (6.2%)
	R	10 (62.5%)	10 (62.5%)	11 (68.7 %)	13 (81.3%)	9 (56.2%)	12 (75%)	9 (56.2%)	13 (81.3%)	11 (68.7%)	12 (75%)
Klebsiella spp. (n=7)	S	4 (57.1%)	3 (42.9%)	0	2 (28.6%)	4 (57.1%)	0	2 (28.6%)	1 (14.2%)	0	0
	I	0	1 (14.2%)	0	0	0	1 (14.2%)	0	0	0	1 (14.2%)
	R	3 (42.9%)	3 (42.9%)	7 (100%)	5 (71.4%)	3 (42.9%)	6 (85.7%)	5 (71.4%)	6 (85.7%)	7 (100%)	6 (85.7%)
Corynebacterium spp. (n=2)	S	2 (100%)	0	0	0	0	0	0	0	2 (100%)	0
	I	0	0	0	0	0	0	0	0	0	0
	R	0	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	0	2 (100%)
Sterptococcus spp. (n=2)	S	0	0	0	2 (100%)	2 (100%)	2 (100%)	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0
	R	2 (100%)	2 (100%)	2 (100%)	0	0	0	2 (100%)	2 (100%)	2 (100%)	2 (100%)
Salmonella spp.	S	4 (100%)	0	0	2 (50%)	2 (50%)	4 (100%)	2 (50%)	0	0	2 (50%)

(n=4)	I	0	0	0	0	0	0	0	0	0	0
	R	0	4 (100%)	4 (100%)	2 (50%)	2 (50%)	0	2 (50%)	4 (100%)	4 (100%)	2 (50%)
Pseudomonas spp. (n=3)	S	1 (33.3%)	2 (66.6%)	0	2 (66.6%)	1 (33.3%)	0	1 (33.3%)	0	0	2 (66.6%)
	I	0	0	1 (33.3 %)	0	0	0	0	0	0	0
	R	2 (66.6%)	1 (33.3%)	2 (66.6 %)	1 (33.3%)	2 (66.6%)	3 (100%)	2 (66.6%)	3 (100%)	3 (100%)	1 (33.3%)

*Abbreviations: P, pattern; S, sensitive; I, intermediate; R, resistant; AK, amikacin; AZM, azithromicin; CFM, cefixime; IMP, imipenem; GM, gentamicin; CTX, cefotaxime; E, erythromicin; CIP, ciprofloxacin; CT, colistin; P, penicillin.

A total 10 classes of antibiotics were used to assess the susceptibility profile of different isolates. Most of the bacterial isolates showed multidrug resistant against 7 and/or more classes (table 6), *Corynebacterium spp*. and *Streptococcus spp* showed 100% resistant against ≥R7 classes. *E.coli*, *Staphylococcus aureus*, *Klebsiella spp.* were 62.5%, 63.5% and 71.4% resistant against ≥R7 classes. *Salmonella spp*. revealed 50% resistant against 5 classes of antibiotics.

Table 6: Multidrug-resistance patterns of bacteria isolated from different body fluids of Coastal patients

Bacterial isolates	**Antibiogram Pattern**							
	R0	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**≥R7**
Staphylococcus aureus **(n=11)**	0	0	0	0	0	1 (9.1%)	3 (27.3%)	7 (63.5%)
E.coli **(n=16)**	0	0	1 (6.2%)	1 (6.2%)	2 (12.5 %)	0	2 (12.5%)	10 (62.5%)
Klebsiella spp. **(n=7)**	0	0	0	0	0	0	2 (28.6%)	5 (71.4%)
Corynebacterium spp. **(n=2)**	0	0	0	0	0	0	0	2 (100%)
Streptococcus spp.	0	0	0	0	0	0	0	2

(n=2)								(100%)
Salmonella spp. **(n=4)**	0	0	0	0	0	2 (50%)	0	2 (50%)
Pseudomonas spp. **(n=3)**	0	0	0	0	0	1 (33.3%)	1 (33.3%)	1 (33.3%)

*Notes: R0- null antibiotic resistance; R1- one class of resistance; R2- two classes of resistance; R3- three classes of resistance; R4- four classes of resistant; R5- five classes of resistance; R6- six classes of resistance; R7- seven classes of resistance.

DISCUSSION

Antibiotic Resistance Pattern

The physicians recommend to prescribe various antibiotics namely, Amikacin, Gentamicin, and Erythromicin to treat a range of illnesses, including urinary tract infections, pneumonia, fever, diarrhea, tonsillitis, vomiting, diphtheria, and typhoid for pediatric patients. Karageorgos and colleagues in a pediatric emergency department in the USA, showing that shorter antibiotic courses have recently been effective for treating conditions like acute tonsillitis, community-acquired pneumonia, and urinary tract infections but there remains some debate regarding the ideal dosing regimen (Karageorgos et al., 2023).

Staphylococcus aureus showed 100% resistant against Penicillin and Ciprofloxacin. A study was carried out in pediatric patients and also found resistant against penicillin (100%) and ciprofloxacin (84.2%) (Gurung et al., 2020 ;Alashoury et al., 2017). A cross-sectional study of patients with wound infections in Bangladesh unveiled the resistancy Cefixime (71.9%) and Colistin (53.9%) (Nobel et al., 2022). The another study in a tertiary care hospital in Nepal, on age group 0-10 years found sensitivity to Gentamycin (83%) (Bhatt et al., 2014). The authors of china reported on children in a hospital that E. coli is increasingly developing antimicrobial resistance and becoming more pathogenic (*New and Highly Infectious E. Coli Strain Resistant to Powerful Antibiotics*, n.d.).

Authors of India unveiled the results on ICU patients that the antimicrobial resistance Ciprofloxacin (100%) and showed no strain as susceptible (Sharma et al., 2023). Similarly, *Corynebacterium spp.* unveiled 100% resistant against all antibiotics except Amikacin and Colistin. In several study on clinical samples in Romania that six strains out of seven of Corynebacterium striatum were resistant to nine out of the eleven antimicrobials tested (Dragomirescu et al., 2020). The main reason behind this resistancy was biofilm formation of these strains (*In Vitro Studies of Non-Diphtheriae Corynebacterium Isolates | IDR*, n.d.)

A study on MDR in *Streptococci* and strategy to nanomedicine that *Streptococci* promote persistent infection, which comprise 80% of microbial infections in humans due to antibiotic resistant and antibiotics are becoming ineffective (Alves-Barroco et al., 2020). Ribosomal mutation and horizontal gene transfer both are the main reasons behind resistancy (Cattoir, 2016). *Salmonella spp.* and *Pseudomonas spp.* both bacteria showed multi-drug resistant. Relevant study were found in author of Iraq on clinical blood specimens (Alkhulaifi & Mohammed, 2023). Except *E.coli* , other 6 bacterial strains showed resistant against ≥R3 classes of antibiotics named multidrug-resistant bacteria. Almost similar results were found in children that nearly two-third (64%) of the organisms were multidrug resistant bacteria (Benzamin et al., 2022). Therefore, physicians should prefer third generation of antibiotics.

Prevalence, Magnitude and Diversity of Antibiotic Resistant Bacteria in Coastal Area

This study revealed samples of body fluids from coastal patient's had 75% positive growth. Higher positive growth (86.6%) was reported in Egypt on body fluids of pediatric patients (Labib et al., 2018) and stay longer at hospital behind this reason. On the other hand, the highest predominant gram negative (-ve) bacteria were 69% in pediatric patients. Almost similar result was on blood stream infection at a clinic in Addis Ababa and was detected gram-negative bacteria 54.5% and gram-positive bacteria 45.43% (Beshah et al., 2022). A high prevalence of *Escherichia coli* indicated coastal waters pose a potential risk for exposure to antibiotic-resistant bacteria (Smalley, 2015). Coastal pediatric patients were mostly infected by Urinary Tract Infections especially at early childhood. The presence of *E. coli* (82.1%) indicates the most common uropathogen in pediatric patients which is the mostly responsible for urinary tract infections (Shkalim Zemer et al., 2024). Results behind this cases were that drinking salt water, wash cloths and sanitary items in dirty and salty water accelerate the rate of UTIs (*Climate Change's Devastating Toll*, n.d.) and are caused by multidrug resistance (MDR) bacteria (Mancuso et al., 2023). One-third of UTI cases in boys during the first year of life, another third between the second and third years, while the peak age of incidence in girls was observed from the third year onward (Banik et al., 2023).

The most invasive bacteria for children were *Staphylococcus aureus* (Cassat & Thomsen, 2021) and was found on new born sepsis patients (Benzamin et al., 2022) which is similar to this study. However, *Klebsiella spp.* (16%) were also predominant bacteria in coastal patients bellow 5 years and cause pneumonia. The fatality of Pneumonia which killed more than 808 000 children under the age of 5 in 2017 and were responsible for 15% of all deaths of children under 5 years (*Pneumonia*, n.d.)

Pneumonia (%) is more prone at infancy stage in coastal area as similar in Bhutan 53.4% (Jullien et al., 2020) because newborns become infected during their passage through the mother's birth canal and infected by *Streptococcus pneumoniae, Klebsiella spp.* and other organisms (*Pneumonia in Newborns - Children's Health Issues*, n.d.).

Males (69%) are more prone to infections than females (31%) in coastal pediatric patients. This increased susceptibility is attributed to factors such as occupational, recreational activities, social roles, and lifestyle (Dias et al., 2022).

Presence of *Corynebacterium spp.* in the body fluids samples of coastal pediatric patients can make an environmental reservoir which could play a direct role in the seafood chain and could pose a potential public health concern (Labella et al., 2013).

CONCLUSION

As a coastal nation, Bangladesh must prioritize the adoption of the blue economy. Environment and various factors are associated with drug-resistant bacteria which has become a potential threat to children, as it leads to infectious diseases. The prevalence of antibiotic-resistant bacteria is high in coastal regions due to shrimp farming activities, saline environments, sewage discharge, and floodwaters. It is crucial to implement eco-friendly technologies like biogenic nanomaterials and microalgae while, reducing the excessive use of antibiotics in human especially pediatric patients.

Limitations of study

Lack of advanced facilities, it was not possible to detect antibiotic resistance encoding genes of the isolates.

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Declaration of interest

It is declared by authors that any conflicts are none to interest.

Authors contribution

Bijon Kumar Saha: Methodology, Sample collection, Lab work, Software analysis, Result interpretations, Writing - Original Draft; Shaila Siddiqua: Conceptualization, Funding acquisition and Supervision; SM Tushar Alom: Validation of microbial results; Ayesha Ashraf: Project administration, Investigation, Reviewing and Editing.

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