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XDR Salmonella- An Emerging Threat in Swabi

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ABSTRACT

Objective: To highlight the threat to effective treatment in more adversely affected areas like Swabi, with XDR Salmonella and stringent implementation of preventive measures to curb the spread of the pathogen.

Methodology: This was a retrospective study conducted from August, 2022 to July, 2023 at Microbiology Laboratory MTI- GKMC/BKMC, Swabi. A total of 2996 blood cultures from patients were submitted at the Microbiology Department, Swabi. All samples were incubated in Bactec 9120 and processed as per standard guidelines, followed by being subcultured on Blood and Mac Conkey agar. On obtaining the non- lactose fermenting colonies, further confirmation of Salmonella species was carried out by biochemical tests followed by final confirmation with API 10S. Antibiotic sensitivity was done by Kirby Bauer disc diffusion technique. Demographic data of the patients was also recorded.

Results: A total of 2996 blood cultures were received in the said duration, out of which 208 were found culture positive for Salmonella species. Out of all the confirmed Salmonella typhi 80% of the isolates were XDR.

Conclusion: Reporting this high incidence of XDR Salmonella species, for the first time from Swabi, KPK is a serious concern, to scrutinize the level of AMR in this region and therefore Pakistan. It is imperative that strict evidence based, tailored as per region, policies need to be implemented strictly to stop the misuse of antibiotics.

Key words: Azithromycin, MDR, Salmonella, XDR.

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Introduction

One of the major public health related threats faced globally is antimicrobial resistance. Ever since the rapidly emerging strains of multidrug and extensively drug resistant Salmonella are further expanding the threat worldwide.

Chloramphenicol was prescribed as the principal drug to treat Typhoid fever till the year 1947, when as a consequence of plasmid-mediated resistance *Salmonella* spp., became resistant to it.[1] Followed by this was the emergence of MDR strains which came to light in 1980s, which were together resistant to the first line antibiotics Ampicillin, Chloramphenicol and Trimethoprim/sulfamethoxazole.[2]

This high prevalence of MDR Salmonella laid grounds for the use of ciprofloxacin, a fluoroquinolone and the third-generation cephalosporin ceftriaxone to treat invasive *Salmonella* infections[3] along with Azithromycin, a macrolide as an alternative.[4] After being endemic in Pakistan for two decades the MDR strains developed further resistance by acquiring a plasmid with a "blaCTX-M-15" extended spectrum β-lactamase resistance genes along with "qnrS" the fluoroquinolone resistance gene.[5]

This lead to the emergence of first case of XDR Salmonella, exhibiting resistance to both fluoroquinolones and third generation cephalosporins along with first-line antibiotics, as reported from Hyderabad a city in the province of Sindh in Pakistan in the year 2016.[6] It was a continuing outbreak which according to WHO[7] had 5274 XDR S. typhi cases from 14 districts of Sindh and later ≥ 10, 000 XDR Salmonella cases.[7] It was not only Pakistan, but many such out breaks were reported from other parts of the world as well.[8,9]

One of the crucial problems being faced after the first case of XDR Salmonella reported from Sindh, Pakistan, there is a persistently increasing prevalence since then. Of further concern would be the development of resistance against the only options left like Azithromycin as cases have already been reported in the near past from Pakistan[10] in the year 2021 as well as from India.[11]

WHO classified the antibiotics into three groups namely "Access", "Watch" and "Reserve", abbreviated as 'AWaRe".[12] It was directed on availability, selection, and prescription of the antibiotics and thus helping oppose the development of antimicrobial resistance.[12] The misuse and abuse of the antibiotics in terms of prescription, dispensing, prevention, self-use, and use in other sectors such as animals, are all very important factors aiding towards AMR quoted as the "biggest threat to modern medicine".

Due to poor accountability for prescribing and selling the antibiotics in addition to lack of knowledge both at the level of physicians and patients, Pakistan is facing a major crisis in terms of antimicrobial resistance and unfortunately is expected to be among the top three developing countries with highest consumption and expenditure on antibiotics.[13,14,15]

Pakistan, is a developing nation, facing lack of availability of resources in their health setup for a huge population and is therefore not at all ready/equipped to face/deal with this endemic.

In order to control this critical situation awareness campaigns about XDR typhoid and the dire need for adapting to good hygiene habits are a need of the hour. Campaigns/trainings particularly focusing on the concerns of the general population about the importance of immunization not only as a part of EPI, but at all ages and their consequent effects in prevention.

Keeping the situation of Salmonella associated AMR, serious efforts need to be put in, in order to produce novel antibiotics. In addition the surveillance system in Pakistan is very poor and needs urgent attention of the Government of Pakistan and the ID experts to enforce such rules to allow strict implementation of antimicrobial stewardship, rather than stepping into a new era of no antibiotics once again.

Methodology

During the research time period, a total of 2996 blood cultures were taken from patients who visited the Teaching Hospital of Bacha Khan Medical Complex, Swabi, at the Microbiology Department. The permission was taken from the institutional review board.

Inclusion criteria: Both the out patients and in patients of all genders and ages were included. Exclusion criteria: Repeated samples from the same patients or patients who already were on antibiotics were excluded from the study.

All samples were incubated in the automated blood culture system, Bactec 9120 and processed according to the standard guidelines by the manufacturer, followed by being subcultured on Blood and Mac Conkey agar. The identification of gram negative organisms was made by standard protocol starting with colony morphology, Gram stain, Catalase test and Oxidase test. Biochemical confirmation was done through Triple sugar Iron test Figure. 1 and final confirmation of the organisms was carried out by the biochemical tests including BioMérieux Analytical Profile Index (API) as shown in Figure 2.

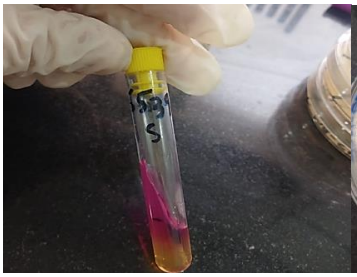

Figure 1: Triple sugar iron for Salmonella typhi

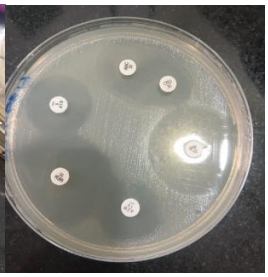

Figure 3. Antimicrobial sensitivity plate.

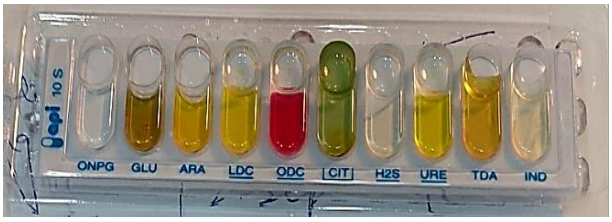

Figure 2. API 10S.

Antibiotic sensitivity was done using Kirby Bauer disc diffusion technique as shown in Figure 3. According to the CLSI 2023[16] the discs applied for sensitivity included Ampicillin, Ceftriaxone, Cefotaxime, Trimethoprim-sulphamethoxazole, Ciprofloxacin, Imipenem, Meropenem and Azithromycin.

Demographic data including age, gender and socioeconomic status were also recorded for diagnosis.

Results

Out of a total of 2996 blood culture samples received, 208 were found positive for Salmonella typhi which is a positivity ratio of 7%.

In the said time period, a total of 208 cases turned out to be culture positive for Salmonella species. 207 were yielded from blood culture where as only one sample of pus also yielded Salmonella typhi. All the isolates were extended-spectrum β-lactamase producers and were sensitive to Imipenem, Meropenem and Azithromycin only.

Among these 208 cases, 58% patients were male while the remaining 42% were females, with the minimum and maximum age limits between 18 months to 56 years.

Table I demonstrates the ward wise distribution out of a total of 208 culture positive samples. Maximum number of the samples was received from the Medical ER i.e.36.5% of the patients, whereas minimum number of patients were received from the Surgical Unit i.e. 1.44%. Age recorded at the time of presentation showed maximum number of patients i.e. 40% were less than 15 years of age as shown in Table I.

Table I: Distribution of the culture positive patients. (n=208)

Ward wise		Age group	
WARD	**No of patients**	**Age**	**No of patients**
Medical ward	31	< 5 Years	01
Pediatrics ward	71	5 to 10 Years	84
ER (Medical)	76	10 to 15 Years	40
Outpatient department	27	> 18 Years	59
Surgical ward	03	> 30 Years	24

Table II: Sources of water for consumption.

Source	%
PHE water source	11%
Wells	35%
Domestic bore hole	47.5%
Hand pump	5%
Reservoir from springs	1.5%

Swabi is a less developed rural area where most of the water for consumption is from domestic bore holes, wells or hand pumps. A very important finding in our study as shown in Table II, indicated the source of water being consumed by the families. It showed that maximum number of patients who were found culture positive for Salmonella species, as per history taken from the patients in the Microbiology Laboratory, were consuming water for all household from the domestic bore holes i.e. 47.5% followed by 35% using wells as the major source.

Major number of culture positive samples yielded Salmonella typhi as the major species responsible for causing Typhoid fever i.e. 177 out of the total 208 followed by the remaining 31 samples which yielded the growth of Salmonella paratyphi, as shown in Table II.

Table III: Species wise distribution of Salmonella species (n=208)

Isolate	(%)
Salmonella typhi	85%
Salmonella paratyphi	15%
Distribution of Resistance shown by Salmonella typhi (n=177)	
XDR	80%
MDR	20%

As shown in Table III, out of the total of 177 Salmonella typhi, 142 were reported as extensively drug resistant whereas the remaining 35 were reported to be multidrug resistant as per CLSI guidelines 2023.[16]

Table IV shows the percentage resistance of the Salmonella typhi against the recommended drugs for both the 207 blood culture samples and one sample received as pus, together making it 208.

It shows that the Salmonella typhi yielded from the pus sample showed 100% resistance to all the antibiotics except Imipenem, Meropenem and Azithromycin and therefore was extensively drug resistant. In addition, it also shows the overall distribution of number of patients received from both OPD and IPD from the two samples blood and pus.

A very positive aspect of this study was to be able to highlight the areas in District Swabi, from which maximum number of cases were received or in other words the areas which need to be kept in strict vigilance for implementation of preventive measures in order to control the spread of XDR Salmonella. As per the collected data maximum number of patients who were reported as culture positive for Salmonella typhi belonged to Gandaf followed by Maner bala and Marghuz showing 43%, 31% and 26% respectively.

Table IV: Total number of patients/samples received from OPD and IPD (n=208) and % resistance shown by Salmonella typhi.

	Number	AMP%	CRO%	CTX%	SXT%	CIP%	IMP%	MEM%	AZM%
OPD	87	94	89	89	99	99	00	00	00
IPD	121	95	82	82	99	99	00	00	00
BLOOD	207	92	54	54	99	99	00	00	00
PUS	01	100	100	100	100	100	00	00	00

Discussion

Endemic in our country and underreported, XDR Typhoid fever is a serious public health concern which is being aggravated further, mainly due to lack of awareness, contamination of the water supplies and poor sanitary conditions[17] and the significance of sending blood culture, before starting the antibiotics, for accurate diagnosis and therefore reduced misuse of antibiotics.

As recommended by the National Action Plan of Pakistan[18], the serological tests were replaced by blood culture tests as blood culture is considered a gold standard[19] not only for accurate diagnosis, but also the relevant antimicrobial sensitivity data provided along with, in order to avoid misuse of antimicrobials.[20]

Since no national guidelines are available for the treatment of typhoid fever, doctors mainly rely on the guidelines provided by Medical Microbiology and Infectious Diseases Society of Pakistan (MMIDSP) which not only enables the physicians to treat better and thus reduce the spread the XDR typhoid.[21]

Based on the results obtained in our study, we are reporting a very high incidence of XDR Salmonella from District Swabi. Our basic concern was to highlight the high level of emerging resistance against different groups of antibiotics in culture positive Salmonella isolates.

In our study the total number of culture positive XDR Salmonella typhi were reported to be 80% of the total culture positive samples, which is a very high percentage. To the best of our knowledge none of the studies in the past have reported such a high incidence from any area of Pakistan. A two year study conducted in Karachi reported the incidence of MDR Salmonella typhi to be 62% and 21%, while 25% and 71% isolates were reported to be XDR, in the years 2017 & 2018 respectively.[22] This percentage was lower than the results obtained in our study for the XDR isolates and for the MDR isolates it was obvious that the level of resistance had increased with a much bigger percentage being XDR isolates, as reported in our study. Similarly, another study conducted in Lahore Pakistan reported MDR and XDR Salmonella typhi in their study to be 24.5% and 46% respectively, again in contrast to our study where the percentage was drastically higher for both.[23]

We reported the disease burden to be greater in males as compared to females which was in accordance with Zakir et.al.,[23] coming up with similar results reporting males to be 61% and females to be 39%. Two other studies also reported results similar to our study, one from Karachi [22] where they reported affected males to be 66% whereas females were reported to be 34%and the other from Islamabad[23] who also reported male predisposition as reported in our study.

As reported by us the major number of patients were below 15 years of age, other studies also reported similar results.[22,23,24] where children were the majorly affected age group.

A very pertinent finding in our study was to identify the water source of all the culture positive patients which revealed that maximum patients were using the bore hole water for all household, drinking and cooking, followed by wells. Many local as well as international studies had repeatedly reported the association of typhoid fever with contaminated water supply/leakages in the sewage pipelines.[25,26] In our study the identification of source of water, was also suggestive of similar associations between contaminated food and water with a greater possibility in rural areas like Swabi. Similarly, another study from Swat also supported the fact of water being contaminated with fecal matter.[27]

As per the results of our study maximum number of patients were from IPD i.e. 121 whereas, the patient who visited OPD were 87 out of a total of 208. This finding was in contrast with the findings in another study who reported more of the Typhoid cases from the OPD being 58% and 42% admitted in the wards.[23]

Although a case report by Ali AM, et al., [10] in the year 2021, have declared the emergence of first case of Azithromycin resistant strain of XDR Salmonella typhi in Pakistan, the antimicrobial susceptibility reported in our study found all the isolates to be sensitive to Imipenem, Meropenem and Azithromycin, similar to the study conducted by Zakir et.al.,[23] and two other studies conducted in Karachi and Islamabad.[22,24]

Recommendations: This study was conducted to collect data and report to the responsible authorities for developing a sustainable solution to this problem. It is imperative that all future policies to incorporate antimicrobial stewardship at institutional level and successful implementation of the National action plan in order to contain the burden of antimicrobial resistance globally.

Conclusion

Reporting this high incidence of XDR Salmonella species, for the first time from Swabi, KPK is a serious concern, to scrutinize the level of AMR in this region and therefore Pakistan. It is imperative that strict evidence based,

tailored as per region, policies need to be implemented strictly to stop the misuse of antibiotics.

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