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SYNOPSIS

INVESTIGATIONS ON ANTIMICROBIAL RESISTANCE: CONTRIBUTIONS OF A CLINICAL RESEARCH NETWORK

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INTRODUCTION

Antimicrobial resistance (AMR) has recently gained global recognition as a priority health care challenge. There is growing consensus that at the rate resistance is developing against existing drugs, and in the face of prevailing deficit in new therapeutic options, emergence and spread of untreatable infections - “a post-antibiotic era” - is a “very real possibility” (1); with a strong potential to reverse the gains of modern medicine in the last century. As such, AMR has been dubbed a global health security threat.

Most antibiotics are prescribed on the basis of collective experience without accurate knowledge of the microbe or its susceptibility to antibiotics. The collective experience will however need to be calibrated based on current data for this approach to sustain. Otherwise, the prevalent practice of empiric treatment that is not further adjusted based on laboratory culture and sensitivity data contributes to poor treatment success and adds to avoidable adverse events. Often, the most potent, reserve or broad-spectrum antibacterial agents are prescribed as a first resort exposing them to early loss of potency with emerging resistance. The secondary effects of depleted drug choices mean that mortality from surgical interventions, cancer chemotherapy, organ transplantation and other services that rely on antibiotic support would rise. The combined effect at national level is higher health care cost as more treatments fail, more toxic alternatives are applied and drug import costs escalate.

There are obvious advantages of empiric therapy as extensive microbiological diagnostics are often not feasible or available on time, even in hospital settings. The rapid spread of drug resistance is forcing antibiotic treatment guidelines to become more and more tailor made with due consideration of the prevailing antibacterial resistance situation in the region or in the health facility and the clinical condition of the patient.

Nowadays, clinicians are finding it increasingly difficult to reliably treat common bacterial infections. In the past, antibacterial resistance (ABR) was mainly seen in hospitals but today community acquired infections with resistant bacteria are increasingly frequent. ABR has reached “alarming levels” globally but the true magnitude is often difficult to assess due to significant gaps in surveillance, lack of standards in methodology and difficulties in generalizability of small data sets and sample sizes.

Different antibiotic resistance patterns of bacteria are currently characterized based on an emerging consensus on terminology. Although these classifications are developed for the purposes of epidemiological monitoring they are also valuable for clinical purposes as well. The definition is based on standard sensitivity testing using clinical cut-off points against an antibiotic. Antimicrobial categories were constructed for organisms or organism groups. An indicator antibiotic can be tested as representative of a category. “A bacterial isolate is considered resistant to an antimicrobial category when it is ‘non-susceptible to at least one agent in a category’ ” (2) due to acquired resistance. Multi-drug resistance (MDR) is defined as non-susceptibility to at least one agent in three or more antimicrobial categories. Extensive drug resistance (XDR) is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories). Pan-drug resistance (PDR) is defined as non-susceptibility to all agents in all antimicrobial categories (i.e. no agents tested as susceptible for that organism) (2). Seventeen antimicrobial categories and agents have been described to define MDR, XDR and PDR for *Enterobacteriaceae* and the same number for *S aureus* in the interim list proposed by the joint initiative of the European and American Centers for Disease Control and Prevention (CDC) (2).

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A high level of resistance was observed against common bacterial agents in a recent systematic review of the literature on AMR in Africa. There was no data for 40% of the countries and the quality of information was poor for the rest (3). A common challenge was the lack of standardization of microbiological identification and susceptibility testing procedures.

Clinical practice guidelines provide recommendations on empirical antibiotic prescribing. A common weakness of such guidelines is that they are often not based on local resistance patterns. Empirical antibiotic treatment was for example discussed in relation to specific microbiologic data in only 16 of 135 (6.4%) guidelines in a recent survey (4). Guidelines “did not routinely consider resistance in their recommendations. Decision-makers should analyse and report the extent of local resistance patterns to allow better decision-making”.

The Ethiopian Food, Medicine and Health Care Administration and Control Authority (FMHACA) 2009 baseline AMR survey report mentions that 61% of health facilities have treatment guidelines (5). The same report underlines however that “no education on the proper use of antimicrobials was given to clients in the health facilities”; that “although the level and training type differs in aggregate, prescribers’ knowledge of antimicrobials was, in certain categories, seriously compounded with the often empirical practices used during treatment; and that “even if there was some awareness of nosocomial infections, little is done by facilities to prevent and contain it” (6).

Contamination, especially with commensal flora is a common challenge in clinical bacteriology. Coagulase negative staphylococci (CoNS) are common contaminants, usually not considered where more virulent pathogens are isolated from specimens. In cultures where CoNS are the only isolates, such as in blood cultures, the decision to consider the isolate as pathogen should be a clinical decision. Laboratory data are however very rarely counter checked against clinical course of disease limiting the quality of data generated in microbiology laboratories. It is generally recommended that at least two separate samples are positive for CoNS before a diagnosis of CoNS infection is made. *In-vitro* antimicrobial sensitivity test reading may be affected by inoculation density, quality of the agar medium, measurement of the inhibition diameter, incubation conditions and quality of sensitivity disks applied.

Synopsis of findings in the current series of articles: In the five studies reported in this Supplement, 674 children under 15 years of age and 1087 women were investigated. A further 288 cases of ocular infections were examined in study six in Addis Ababa.. Patients presenting with blood stream, urinary tract, gastrointestinal and tissue infections were enrolled in hospitals in Addis Ababa, Dire Dawa, Gondar, Jimma and Hawassa. A single blood, urine, swab or stool sample was cultured per patient. From a total of 2049 cultures, 512 bacterial isolates were tested for antibiotic susceptibility.

The most frequent isolates were *E. coli* (137), *S. aureus* (108), CoNS (87) and Klebsiella (62). Shigella (22), Salmonella (7) and *E. coli* O157:H7 (13) were predominant in stool whereas *S. aureus* (63), CoNS (54) and *E. coli* (18) dominated in conjunctival swabs. Pseudomonas (14) was mostly found in blood (6) whereas *E. coli* (55) and Klebsiella dominated in urine.

The most effective antibiotics against *S. aureus* among those tested were clindamycin (92% susceptible), erythromycin (88%), gentamicin (85%) and ciprofloxacin (85%). Sensitivity was lowest to penicillin (7%), tetracycline (12%), ampicillin (38%) and cefoxitin (33%). *S. aureus* isolates from ocular swabs were generally more susceptible to antibiotics (except to penicillin and tetracycline) than isolates from blood or other specimens.

Coagulase negative staphylococci were reported frequently from blood cultures and conjunctival swabs. Their sensitivity pattern appeared to mirror those of *S. aureus*. Both responded best to gentamicin (86%), erythromycin (81%), chloramphenicol (77%) and ciprofloxacin (72%). The least effective antibiotics against CoNS were penicillin (3%), tetracycline (19%) and ampicillin (36%). Isolates from eye swabs were resistant to tetracycline (only 6% sensitive) even more so than CoNS isolates from elsewhere (62% sensitive). CoNS isolates from conjunctival swabs were more sensitive to cotrimoxazole (59% vs 9%) and erythromycin (89% vs 46%) than CoNS isolates from elsewhere.

The most effective antibiotics against *E. coli*, except for amikacin (24/24 sensitive), norfloxacin (22/24), imipenem (49/54), azithromycin (13/13) and tazobactam (13/13), were ciprofloxacin (67% sensitive) chloramphenicol (65%) and gentamicin (55%). The least effective antibiotics against *E. coli* were ampicillin (13% sensitive), tetracycline (33%), and cotrimoxazole (36%). Blood isolates appeared to be more frequently sensitive to co-trimoxazole than urinary isolates (85% vs 33%).

Urinary isolates of *E. coli* were in general more frequently resistant to antibiotics than blood isolates (except against ampicillin, amoxicillin and tetracycline to which most *E. coli* were resistant anyway). *E. coli* from swabs were frequently resistant to ampicillin (11% sensitive), tetracycline (11% sensitive) and co-trimoxazole (28% sensitive). Meropenem resistance of 42% (23/55) was reported from female urinary tract infections in Jimma.

The sensitivity pattern of *Klebsiella* isolates mirrored closely those of *E. coli* with below 50% sensitivity to commonly used antibiotics including gentamicin, ceftriaxone, co-trimoxazole and amoxicillin-clavulanic acid. Sensitivity to ampicillin was only 5% and that to co-trimoxazole 31%.

What the findings mean in the context of Ethiopia

In relation to clinical practice guidelines: Guidelines for drug treatment in Ethiopia issued by the Drug Administration and Control Authority (DACA), subsequently FMHACA, in 2010 (7) recommend cotrimoxazole as first line drug for the treatment of uncomplicated urinary tract infections, with norfloxacin or amoxicillin as alternatives. The most frequently isolated bacteria in UTI was *E. coli*. The pooled sensitivity to cotrimoxazole was only 33% (with resistance at 42% in Gondar and 80% in Jimma). Amoxicillin resistance was 50% in Gondar and 80% were resistant to amoxicillin-clavulanic acid in Jimma. Resistance to norfloxacin was however low in Gondar (9%). Ciprofloxacin resistance was 56% in Jimma. The above data, although not based on large sample sizes, suggest that first line recommended agents for UTI are inappropriate, and updated guidelines are needed.

First line recommendation for septicemia in Ethiopia (7) is parenteral ampicillin and gentamicin. The alternative treatment recommended is gentamicin with ceftriaxone or with cloxacillin and ceftazidime. Focusing on *E. coli* and *S. aureus* as the most frequent isolates from blood stream infections, while *E. coli* remains frequently sensitive to gentamicin (85%), ampicillin appears to be a poor choice for coverage against *S. aureus*, with only 38% of isolates sensitive. This suggests that this regimen for sepsis in general may be outdated. These observations again reinforce the need to update national guidelines with comprehensive recommendations.

For bacterial conjunctivitis, the guidelines recommend topical tetracycline or erythromycin as first choice and chloramphenicol and gentamicin as alternative therapies with ceftriaxone as the reserve drug of choice.

The *E. coli* isolates from conjunctival swabs were 89% resistant to tetracycline but 94% sensitive to gentamicin and 72% to chloramphenicol. Isolates of *S. aureus* from conjunctival swabs were 87% sensitive to erythromycin, 98% to gentamicin and 73% to chloramphenicol but only 5% to tetracycline. Evidently, tetracycline seems to have lost efficacy in the treatment of common bacterial conjunctivitis.

Monitoring of antibiotic resistance trends needs to take into consideration the spatial, temporal and methodological aspects of data collection. Proportions of sensitive and resistant strains of a pathogen such as *E. coli* vary in different settings based on strain subtypes, local selection pressure, nature of infection, host immunity, predisposing factors such as types of medical interventions, among others. Comparison of the pooled current data set against previous reports lumps together a number of distinct variations and is therefore simplistic. Its purpose can only be to provide a general idea of levels of resistance and cannot serve as a guide to clinicians for individualized treatment. For example, we have suggested above that the current recommendation of ampicillin-gentamicin is not ideal empiric coverage for sepsis due to a common organism such as *S. aureus*; however, ampicillin would be a better choice for empiric coverage of sepsis where the underlying source infection were more likely due to for example Streptococcal species, even though those organisms were not common in our studies. Clinicians should be supported with basic bacteriology services to ensure that treatment is tailored to the specific condition and need. Nevertheless, in the absence of better evidence, important information can be deduced from such pooled data for the purposes of program use.

In relation to antibacterial surveillance: The priority pathogens selected by the Ministry of Health for surveillance in Ethiopia are *E. coli*, *Klebsiella pneumoniae* and *S. aureus* obtained from urine and wound specimens and all carbapenem-resistant organisms, regardless of specimen type (8).

According to the DACA national baseline AMR surveillance report, resistance of *E. coli* to ampicillin averaged at 70% for the five years to 2008 (3399 isolates, 2004-2008; range: 65% -74%). The current figure of 87% resistance suggests further loss of efficacy of ampicillin against *E. coli* in the last 10 years. Resistance to tetracycline, 67% in current series, is slightly lower than the baseline estimates of 75% (n=2799, range: 65% -90%).

Resistance levels of *S. aureus* isolates (from sites other than conjunctival swabs) against erythromycin at 66% in current series is similarly lower than the 87% reported in the survey (n=495, range: 78-94%). However, resistance to ceftriaxone, 45% in current series is higher than the 20% (n=583; range: 12-47%) estimate in the baseline survey.

Resistance levels of *Klebsiella* against ampicillin (95% vs 70-97%) and tetracycline (59% vs 55%-88%) fell within the range of baseline survey report in this series but current estimates from this series were higher for *Klebsiella* resistance against cotrimoxazole (69% vs 43-58% range in baseline survey).

The pooled overall sensitivity of the 137 *E. coli* isolates from all specimens in this Supplement was surprisingly close to the pooled percentage from the meta-analysis of the literature reported from Ethiopia between 2007-2017 involving 2635 isolates (9): resistance to ampicillin 86.9 vs 83.8%, ceftriaxone 49 vs 30.2%, chloramphenicol 35.4 vs 35.3%, ciprofloxacin 32.9 vs 27.6%, gentamicin 45.3 vs 35.8%, tetracycline 67.3 vs 67.2%, cotrimoxazole 64.2 vs 57.5%.

The average proportions of antibiotic resistance among the 108 isolates of *S. aureus* in the studies reported in this Supplement were in most cases close to the pooled estimates of *S. aureus* isolates from 4750 patients presented in a recently reported meta-analysis of the literature from Ethiopia (10) (ampicillin 62 vs 75%, ciprofloxacin 15 vs 19%, chloramphenicol 37 vs 37%, gentamicin 15 vs 26%, cotrimoxazole 49 vs 47%, clindamycin 8 vs 24%, erythromycin 12 vs 41%; all falling within the 95% confidence intervals calculated for the individual antibiotics compared to the pooled estimate). Differences were observed in the case of ceftriaxone 45 vs 34%, tetracycline 88 vs 62%, penicillin 93 vs 76% and ceftioxin 67 vs 27%, in each case showing higher resistance in the current series than in the meta-analysis. Important to consider in this regard is the small number of isolates tested in the current series of articles.

DISCUSSION AND CONCLUSIONS

The collected data provide a good insight into the prevailing trend of antimicrobial resistance in the health facilities investigated. The main value of the studies is obviously in their application at the respective health facility where the data were gathered from. The articles have provided relevant recommendations on what measures need to be taken to improve antimicrobial use practices. The data will provide baseline information for monitoring trends.

The impact of the work would be maximized if antimicrobial stewardship programs are implemented in the health facilities to coordinate interventions that ensure appropriate use of antimicrobials (including selection of the optimal antimicrobial regimen, dose, duration of treatment and route of administration) (11). Such programs have shown benefits in improving clinical outcomes, reducing toxicity, cost, drug resistance and hospital stays for inpatients. A major task of such a program in our setting would be ensuring availability and smooth running of a quality assured bacteriology service that works closely with clinicians. Successful programs had to overcome several challenges including of funding, human resource, information technology, prescriber opposition, and lack of commitment from management due to poor awareness or prioritization (12).

Further analysis of the data from the studies in this Supplement would provide more insight into the epidemiology of antibacterial resistance in Ethiopia. Several research questions would arise from a more in-depth pooled analysis that would compare these data against previous information in the literature. Molecular investigation of the isolates can provide answers related to transmission pathways and identify the most important strain types or clones to focus on.

The supplement is a result of coordinated research among several institutions involving academic departments, university hospitals and health centres. This collaborative work on antimicrobial resistance has mainly focused on clinical research questions. The recommendations will improve clinical practice where applied. The laboratory capacity strengthened in the process of raising these data is expected to continue supporting further bacteriology research in the respective facilities enabling further exploration of the antimicrobial resistance problem in the network.

Sequential data of drug resistance from prospective clinical and surveillance studies can replace the prevailing practice of partial and fractional publication by region, health facility, period of time, population, bacterial type or antibiotic. There are several challenges in how to rapidly identify resistant pathogens even in advanced centres of the global north that have access to latest technologies (13). A strong national clinical research network can attract strong international partners for mutually beneficial antimicrobial research.

In addition to the types of clinical investigations reported here, behavioral, social and economic studies are required to understand and tackle the key factors driving antibiotic resistance. Of particular concern is the rate of resistance among bacteria that cause common health problems associated with community acquired infections.

Unfortunately, community acquired infections are under-represented in reports of antimicrobial surveillance which often focus on hospital acquired infections or on severe illnesses resistant to first line treatment. This calls for expansion of the network to include relevant disciplines and consolidate a partnership that will in due course generate new knowledge and at the same time provide the evidence base for improved practice and policy recommendations.

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