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Surveillance of antibiotic resistance determinants in commensal and environmental bacteria from international sources

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Abstract

Background: Commensal and environmental flora form a large reservoir of mobile genetic elements bearing genes for antibiotic resistance which can transfer resistance to other bacteria, including pathogens. APUA, in conjunction with the National Biodefense Analysis and Countermeasures Center, has undertaken an international surveillance of the reservoirs of antibiotic resistance (ISRAR) in commensal bacteria to track geographically, and eventually temporally, the emergence and spread of resistance genes through its online Reservoirs of Antibiotic Resistance (ROAR) database.

Methods : Protocols were developed to optimize isolation of 9 targeted bacterial genera from healthy animals and environmental sites. Isolates obtained from 8 APUA country chapter (Bangladesh, Georgia, India, Turkey, Uganda, S. Africa, S. Korea, Vietnam) laboratories were speciated (API) and susceptibility tested (E-test) using appropriate classes of antibiotics.

Results: There was 85% recovery of target species. Between 2008-2010, 1079 total isolates: *E. coli*, (389) *Staphylococcus* (329),*Streptococcus/ Enterococcus* (79), *Salmonella* (121), *Pseudomonas* (49), *Acinetobacter (49) Aeromonas* (33) and *Stenotrophomonas* (50) were isolated from healthy animals (807) water/sewage (193), plants (36) and soil (43). >70% of all isolates expressed resistance to one or more antibiotics; ~30% demonstrated multidrug resistance (≥2 drugs). Resistance patterns were different among environmental sources and geographic sites.

Conclusions: This study has revealed significant differences in the frequency of particular antibiotic resistance markers in bacteria isolated from geographically diverse environmental sites.

Introduction

Antibiotic resistance—one of the top five global health problems—seriously compromises the medical treatment of bacterial infectious disease. Studies to date have focused largely on drug resistance in disease-causing bacteria. Commensal (non-disease causing) and other environmental bacteria comprise a very large reservoir of genes that encode resistance to antibiotics (1). These can be transferred among commensal / environmental bacteria, as well as human pathogens (2).

In November of 2009, the US National Strategy for Countering Biological Threat established a goal to build international capacity to collect and detect infectious diseases threats worldwide. APUA, along with governmental partners at the National Biodefense Analysis and Countermeasures Center (NBACC), has been proactive in establishing an extensive international surveillance system to better understand the sources of the antibiotic resistance threat and potential interventions. This public-private partnership combines APUA's global public health resources with those of the national biodefense agency to monitor global patterns of resistance—an objective neither organization could have accomplished alone. This study is the first of its kind to track and evaluate the environmental load of resistance markers in diverse geographic sites.

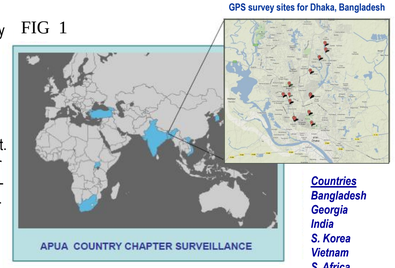
Methods

Through the use of APUA-derived protocols that incorporate selective agars, gram stains and simple standard biochemical tests, commensal/environmental isolates of 9 bacterial genera are being collected on a continuing basis from 7 APUA chapter network countries worldwide.

GPS mapping technology is being employed to record and map collection sites for all data. (Fig1). Isolate sources included fecal, skin and nasal specimens from healthy animals, soils, plants, and fresh and polluted waters.

Following shipment to Tufts University, the presumptively identified isolates are confirmed by API biochemical test strips and minimal inhibitory concentrations (MICs) are determined to selected antibiotics by E test. CLSI standards are used for evaluating MIC data. All isolate data are being incorporated into APUA's online Reservoirs of Resistance (ROAR) database for trend analysis (www.ROARproject.org).

FIG 1



Results

Confirmation of bacterial identities revealed ~85% recovery of target species from all isolates recovered by the site laboratories. This analysis focuses on the first 600 isolates of a representative gram-negative (*E. coli)* and gram-positive (*Staphylococcus* spp.). The data reveal different frequencies of resistance among countries and continents:

E. coli

- Signficant frequencies of resistance (≥10% of the total isolates) were widespread and most predominant among the older drugs (tetracycline, ampicillin, trimethoprim, sulfamethoxazole). High-level resistance (≥50%) was also common, particularly to sulfamethoxazole. (Table 1).
- 61% of isolates showed some intermediate or full resistance to at least one antibiotic. 31% were multi-drug resistant (2 - 8 drugs) (Fig 2).

Staphylococcus spp.

- Significant frequencies of resistance were comparatively less prevalent than in *E. coli.* High level frequencies (≥50%) appeared only rarely and only to penicillin. Resistance to newer drugs was generally <10%. (Table 1)
- 80% of *Staphylococcus* spp. showed some level of intermediate or full resistance. 49% were multidrug resistant (2 - 6 drugs) (Fig 2).

TABLE 1. ANTIBIOTIC RESISTANCE FREQUENCIES (%) IN 8 GLOBAL SITES

E. coli	#	cip	gen	chl	tet	cep	amp	trim	sulf	strep
Bangladesh	14		0	0	42	29	23	36	92	7
Georgia	67	0	0	5	15	1	5			
India	89	6	4	0	20	5	12	17	33	0
Turkey	120	1	0	4	20	8	18	9	63	9-14
S. Africa	10		0	0	50	0	10	10	50	10
Vietnam	12		17	68	91	0	25	58	92	25
Uganda	52	12	1	14	62	10	54			
S. Korea	10	40	30	30	60	10	50			

Staphylococcus spp.*	#	pen	cip	gen	oxa	chl	tet	erm	cep	amp	trim
Georgia	21	29	5	0	0		19	9.5	0		
India	93	33	4	0	2		2	3	0	38	
Turkey	122			9		6	3	3	0	28	17
S. Africa	7			0		0	14	14	0	0	29
Vietnam	4			25		25	25	25	0	25	50
Uganda	18	30	0	0	0		14	24	0		
S. Korea	10	90	0	10	0		0	0	0		

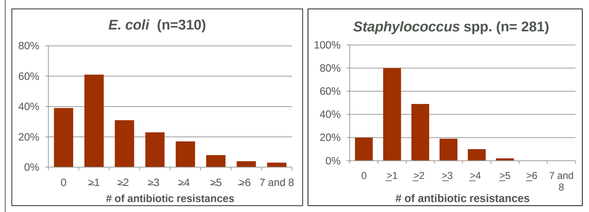
≥10% of total isolates are fully resistant

≥50% of total isolates are fully resistant

cip= ciprofloxacin; gen= gentamicin; chl= chloramphenicol; tet= tetracycline; cep=cephalothin; amp=ampicillin; trim=trimethoprim; sulf=sulfamethoxazole; strep=streptomycin

*species= *aureus/intermedius(63), auricularis, caputus, cohnii, chromogens, epidermidis, hemolyticus, hominus/chromogens, lycus, lentus, saprophyticus, sciuri, simulans, xylosus*

FIG 2. Frequencies of multidrug resistance



- Median MICs to certain drugs were markedly different from country to country (especially ampicillin, ciprofloxacin, and tetracycline for *E. coli*) (Fig 3a) and gentamicin, ampicillin, and tetracycline for *Staphylococcus* (Fig 3b).
- Scatter plots are being used to evaluate the different ranges and modes of MIC distribution among commensal/environmental bacteria (Fig 4).

FIG 3a. Median MICs: ***E. coli***

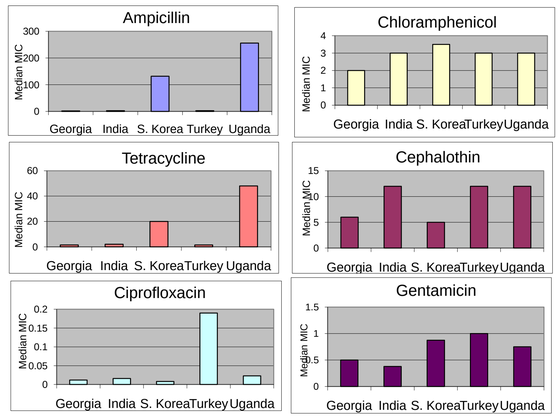


FIG 3b. Median MICs: ***Staphylococcus* spp**

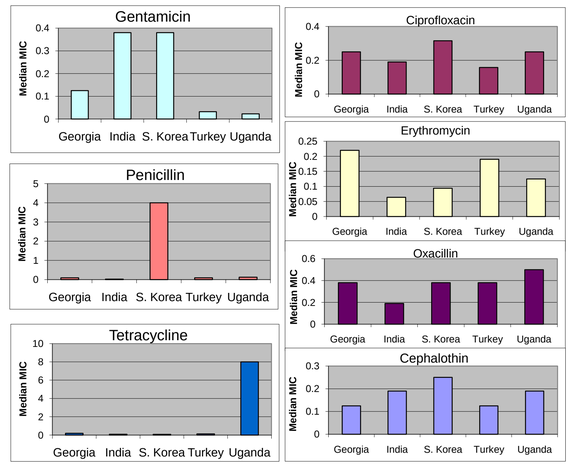
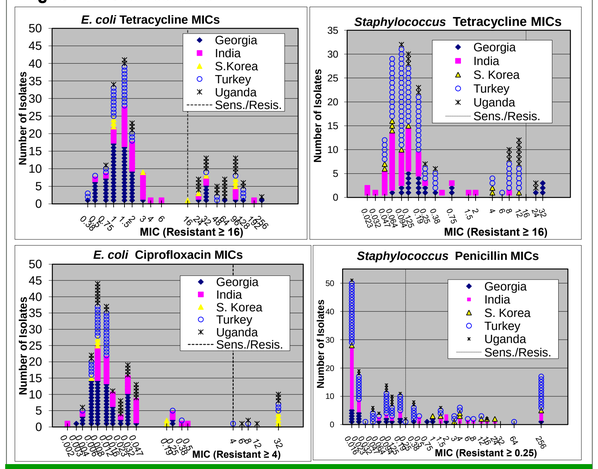


Fig 4. MIC Distributions



Conclusions

Commensal and environmental bacteria are reservoirs for genes encoding resistance to commonly used antibiotics. The data support prior findings in the American and European continents of resistance in non-disease-causing bacteria.

➢ Resistance was more common among *E. coli* than among *Staphylococcus* spp and significant frequencies (>10%) were consistently found to older drugs, e.g., tetracycline, ampicillin, trimethoprim, and sulfamethoxazole.

➢ Different antibiotic use and distribution patterns may be responsible for the varying environmental frequencies of resistance found.

➢ The findings support ongoing collection of isolates for trend analyses and for genetic studies to determine the basis for these significant differences.

References

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