Mycobacterial Diseases
ISSN: 2161-1068

Yeboah-Manu, Mycobac Dis 2012, 2:3
DOI: 10.4172/2161-1068.1000107

Review Article Open Access

Drug Susceptibility Pattern of Mycobacterium Tuberculosis Isolates From Ghana; Correlation with Clinical Response

Dorothy Yeboah-Manu[1]*, Adwoa Asante-Poku[1], Kobina Assan Ampah[1], Grace Kpeli[1], Emelia Danso[1], Kwaku Owusu-Darko[1] and Frank Addae Bonsu[2]

[1]Noguchi Memorial Institute for Medical Research, Legon, Ghana
[2]National Tuberculosis Program, Ghana Health Service, Ghana

Abstract

Background: The worldwide emergence of drug resistant forms of the *Mycobacterium tuberculosis* complex, the causative agents of tuberculosis (TB), has the potential to make this important human infectious disease which is generally treatable, virtually incurable if not controlled. Our aim therefore was to determine the *in vitro* drug susceptibilities of *M. tuberculosis* complex isolates and to correlate this with the clinical response of patients from whom the isolates were obtained.

Methodology: Sputum samples obtained from smear positive cases were cultivated on Lowenstein Jensen (LJ) medium. The susceptibilities to isoniazid (INH), rifampicin (RIF), streptomycin (STR) and ethambutol (EMB) were determined by the indirect proportion method, following isolate identification. Drug susceptibility of the isolates was then correlated with the individual clinical outcomes.

Results: One hundred and twenty one *M. tuberculosis* complex isolates were analyzed in this study. One hundred and nine (90.08%) and 12 (9.92%) were from new and previously treated cases respectively. Thirty-eight (31.40%), 18 (14.88%), 8 (6.61%) and 4 (3.31%) were resistant to STR, INH, RIF and EMB respectively. Forty seven (38.84%) of the tested isolates was resistant to at least one drug. Thirty one (25.62%) of the isolates were mono-resistant to one of the drugs; 24 (19.83%), 3 (2.48%), 3 (2.48%) and 1 (0.82%) to STR, RIF, INH and EMB respectively. Poly-resistance to STR/RIF, STR/INH and EMB/INH was observed in 2 (1.65%), 10 (8.26%) and 1 (0.82%) isolates respectively. Three (2.48%) of the isolates were multi-drug resistant (MDR) and of these, 2 were resistant (1.65%) to all the tested drugs and one was resistant to RIF and INH. Correlating the drug susceptibility with the clinical outcome of 79 cases including 2 MDRs, we found that among our study population, the clinical outcome depended on whether the isolate was sensitive or resistant to RIF ($p<0.0005$).

Conclusion: A high level of primary drug resistance was observed, particularly to STR and INH, among the *M. tuberculosis* complex isolates in our study population and that treatment outcome depends mainly on the susceptibility of RIF.

Background

Tuberculosis (TB) continues to be a major public health problem in the world. It is estimated that one person dies every 15 seconds of TB and the World Health Organization (WHO) indicates that more than 9 million cases occurs annually with a mortality of 2 million [1]. The Directly Observed Treatment Short Course (DOTS) strategy, which allows patients to take their daily drugs under observation, thereby improving treatment compliance, is known to be increasing the number of people being cured of TB [2]. A major challenge to this strategy in TB control globally is the incidence of strains of the *Mycobacterium tuberculosis* complex, the causative agent of TB, that are resistant to the first line drugs, especially Rifampicin (RIF) and Isoniazid (INH) [3]. Individuals infected by such strains are not able to be cured by the DOTS treatment strategy and also make case management more complicated and expensive. There are an estimated 460,000 multidrug-resistant TB (MDR-TB) cases each year and approximately 25,000 of these cases are expected to have extensively drug-resistant TB (XDR-TB). MDR TB requires 18–24 months of treatment with expensive second line drugs, some of which are injectable agents. The cure rate is much lower than for drug susceptible TB, only around 60% [3-5].

A crucial strategy for reducing the spread of MDR-TB is rapid detection of drug resistance followed by prompt and effective therapy [3-5]. The conventional laboratory diagnosis of drug resistant TB requires a viable, pure culture of *M. tuberculosis* complex organisms, followed by further cultivation on drug containing solid medium. The slow growing nature of the species of the *M. tuberculosis* complex makes conventional drug susceptibility testing a very slow and demanding process. The time between primary isolation and final Drug Susceptibility Testing (DST) result is usually weeks and can be more than two months [6-8]. Recent advances in the molecular detection of mutations that are associated with resistance to certain drugs and can be performed using DNA extracts prepared directly from sputum specimens can often provide results on the same day [9]. However this is not possible in countries with limited resources, where TB diagnosis relies mainly on sputum smear microscopy. Thus routine surveillance of the kind and level of resistance is very important. This will help in planning treatment regimens [8].

Ghana has an annual TB incidence rate of 203/100,000 population,

***Corresponding author:** Dorothy Yeboah-Manu, Noguchi Memorial Institute for Medical Research, Legon, Ghana, Tel: 00233-208123882; E-mail: dyeboah-manu@noguchi.mimcom.org

Received March 02, 2012; **Accepted** April 05, 2012; **Published** April 07, 2012

Citation: Yeboah-Manu D, Asante-Poku A, Ampah KA, Kpeli G, Danso E, et al. (2012) Drug Susceptibility Pattern of Mycobacterium Tuberculosis Isolates From Ghana; Correlation with Clinical Response. Mycobac Dis 2:107. doi:10.4172/2161-1068.1000107

and ~50,000 new TB cases occur every year [1]. Current TB control measures in Ghana (like in most other developing countries) are primarily based on sputum smear microscopy, which has the ability to detect less than 50% of all TB cases [10]. Hence the real TB burden in Ghana is likely to be substantially higher than the official WHO estimates. Importantly, because of the lack of appropriate laboratory infrastructure, DST is not routinely performed in Ghana, and the extent of drug-resistant TB is not entirely known. The two main objectives of this study were 1) to determine the *in vitro* drug susceptibilities of isolates obtained from TB patients 2) to determine whether the clinical responses correlate with the *in-vitro* drug susceptibilities of cases, especially MDRs.

Methods

Specimen and data collection

This was a cross-sectional analytical study in which all consecutive individuals, diagnosed with smear-positive pulmonary TB cases were enrolled between October 2007 and July 2009. A total of 121 isolates from 121 TB cases attending three different health facilities in four different districts were included in this study. The National Tuberculosis programme was responsible for selecting the districts. One district has a refugee population of over 20,000. The other two districts were selected based on the capacity of the laboratory technicians to provide the specimens required and good track record of TB data documentation. Using a designed questionnaire, data on clinical characteristics, previous illness due to TB, previous therapy received family history of TB and standard demographic data including age, sex, and residential address were obtained from each participant. Two previously analysed smear-positive sputum samples from each participant were then kept at the diagnostic centre/laboratory after addition of an equal volume of 1% cethylpyridium chloride (CPC): 2% sodium chloride decontaminant. All collected samples were stored, tightly capped, in an enclosed container and transported to the Noguchi Memorial Institute for Medical Research (NMIMR) for in-depth analysis within one week of collection. Approval for this study was obtained from the Institutional Review board of the NMIMR.

Isolation of *Mycobacterium species*

All collected sputum samples were inoculated into 4 Lowenstein-Jensen (LJ) slants: 2 containing glycerol and the other 2 containing pyruvate. The inoculated slants were incubated at 37°C and the culture tubes were observed for mycobacterial growth. All mycobacterial isolates was identified using biochemical methods such as susceptibility to p-nitro benzoic acid (PNB) and to thiophene carboxylic acid hydrazide (TCH), pyrazinamidase activity (PZA), nitrate reduction, niacin production; and detection of IS6110 by PCR analysis [12,13]

Anti-TB drug susceptibility testing

The susceptibilities of all identified *M. tuberculosis* complex isolates to INH (Sigma, I3377) (0.2 µg/ml), RIF (Sigma, R3501) (40 µg/ml), streptomycin (STR, Fluka, 85880) (4 µg/ml), and ethambutol (EMB Sigma E4630) (2 µg/ml) were determined by the indirect proportion method on LJ slants, as described previously [6].

Briefly, 1-2 McFarland bacterial suspensions were prepared in 5 ml screw-cap tubes containing glass beads (diameter 3.0 mm) in sterile distilled water. The suspensions were homogenized on a vortex mixer for 1 min and left to stand for at least 15 min to allow aerosol created during vortexing to settle. Serial 10-fold dilutions up to $1/10^4$ were prepared with sterile distilled water. $1/10^2$ dilutions were then used to inoculate drug containing media in duplicate while both the $1/10^2$ and $1/10^4$ were used to inoculate drug-free controls respectively. The tubes were incubated overnight at 37°C in a slanted position with loosened caps to allow the cells to settle on the medium and residual liquid to evaporate. After overnight incubation, the screw caps were tightened and the tubes were further incubated at the same temperature in an upright position. The initial reading of the tubes was performed on day 28 of incubation, while the final reading was done after 40 days of incubation. Drug resistance was expressed as the proportion of colonies that grow on drug containing medium to drug-free medium and the critical proportion for resistance was 1% and intermediate resistance is between 1-10% for all drugs [6].

Definitions

Multidrug resistance (MDR) was defined as resistance to at least INH and RIF. Other cases were categorized as follows: Drug sensitive – susceptibility to all of the drugs tested, monoresistance – resistance to only 1 drug; polyresistance – resistance to two drugs excluding the INH:RIF combination and pan resistance- resistance to all four tested drugs [5].

Assessment to Treatment Outcome

Cases were followed during treatment and the final outcome, as recorded by the treating facility, was compared with the drug susceptibilities of the cultured isolate in our laboratory.

Cases were defined as previously

A new patient was defined as a TB patient who either had no prior anti-TB treatment or was treated with anti-TB drugs for less than 1 month [11].

A defaulter was defined as a patient who interrupted his treatment for more than 2 months after having received anti-TB treatment for at least 1 month.

A relapse was considered an individual who became smear positive again after having been treated for TB and declared cured after the completion of treatment.

A treatment failure case was considered a patient who began treatment for smear-positive TB but who remained smear positive or became smear positive again 5 months or later during the course of treatment.

Treatment was completed if the patient was converted to smear negative at month 5, completed treatment but did not produce sputum on completion to be declared cured.

A case was considered cured if the patient completed treatment and maintained smear negativity on smear microscopy examination after treatment.

Data Analysis

All collected records were entered into a Microsoft Access database and exported to Excel for analysis. Data were expressed in means ± SD and ranges. The proportions of resistance to individual drugs and to different drug combinations were tabulated. Also, resistant cases were differentiated as primary (being treated for TB for the first time) or acquired (previously treated). Student's independent samples t tests for numeric variables and chi square test for categorical variables. All significant levels were based on a p value less than 0.05.

Results

Patients characteristics

The majority of patients were male, 82 (67.77%) and 39 (32.23%) were female. The age range was 8-88 years, arithmetic mean 37.25, modal class is 26 and median age of 36. The median age among female subjects was 30 (SD 8.3) with a range of 8-88 years while that of the males was 34 (SD 8.6) with a range of 18–62 years. Among them were 10 (10.26%) refugees who live in the refugee camp at Budumbura. Patients sought healthcare after one month to 5 years of productive cough, with mean diagnostic delay of 6 months. All the cases were pulmonary sputum smear positive and confirmed by culture; and of these 109 (90.08%) were recorded as new cases with no history of previous treatment, while 12 (9.92%) had received previous treatment for TB.

Resistance profile

One hundred and twenty-one isolates comprising of 99 *M. tuberculosis* and 22 *M. africanum* West African genotype were analysed in this study. The resistant profile of tested isolates is depictured in table 1. Of the 121 isolates, 71 (58.68%) were susceptible to STR, 12 (9.92%) were intermediately resistant and 38 (31.40%) were resistant. One hundred and two (84.30%) of the isolates, 1 (0.82%) and 18 (14.88%) were susceptible, intermediate resistant and resistant to INH respectively. From the results of the 121 isolates, 113 (93.39%), and 8 (6.61%) showed susceptibility and resistance respectively to RIF. One hundred and seventeen isolates (96.69%) were susceptible and 4 (3.31%) were resistant to EMB.

Thirty one (25.62%) of the isolates were mono-resistant to either one of the drugs; 24 (19.83%), 3 (2.48%), 3 (2.48%) and one (0.82%) to STR, RIF, INH and EMB respectively. Poly-resistance to STR/RIF, STR/INH and EMB/INH was observed in 2 (1.65%), 10 (8.26%) and 1 (0.82%) isolates respectively. Three (2.48%) of the isolates were MDR and of these, 2 (1.65%) were pan-resistant and one was resistant to RIF and INH. Thus in all 47 (38.84%) of the tested isolates was resistant to at least one drug.

Twelve isolates were obtained from previously treated cases; 6 (50%) were susceptible to all drugs; one each (8.33%) intermediate resistant and mono-resistant to RIF respectively, 2 (16.67%) were poly-resistant to STR/INH and the remaining 2 (16.67%) of the isolates were pan-resistant.

Drug susceptibility and outcome of patients' treatment

All patients involved in this study were treated with the standardised 6-month short course therapy regimen with INH and RIF as the main drugs. We were able to follow 79 (65.23%) of the cases during treatment and of these, 40 (50.63%) were cured, 23 (29.11%) completed treatment, 8 (10.13%) defaulted, 4 (5.06%) died, 2 failed (2.53%) and 2 (2.53%) relapsed.

Anti-TB drug	Number of isolates tested	Susceptible n (%)	Intermediate Resistant	Resistant
STR	121	71(58.68%)	12 (9.92%)	38 (31.40%)
INH	121	102 (84.30%)	1(0.82%)	18 (14.88%)
RIF	121	113(93.39%)	0	8 (6.61%)
EMB	121	117(96.69%)	0	4 (3.31%)
STR/INH	121			10 (8.26%)
STR/RIF	121			2 (1.65%)
INH/EMB	121			1(0.82%)
MDR	121			3 (2.48%)

n = number

Table 1: The susceptibility patterns of tested isolates to first-line anti-TB drugs from Ghana.

Resistance	Female n(%)	Male n(%)	P VALUE
STR	9(23.08%)	15(18.29%)	0.3065
INH	1(2.56%)	2(2.44%)	0.9447
RIF	2(5.13%)	1(1.21%)	0.1085
EMB	1(2.56%)	0(0%)	0.1422
STR/INH	4(10.26%)	6(7.32%)	0.3798
STR/RIF	1(2.56%)	1(1.21%)	0.4422
INH/EMB	1(2.56%)	0(0%)	0.1422
MDR	2(5.13%)	1(1.21%)	0.1085
TOTAL	21(53.84%)	26(31.70%)	<0.0001

Table 2: The distribution of drug resistance phenotypes between males and females observed among study participants.

Completed cases: The *M. tuberculosis* complex isolates from 15 (65.22%) of the completed cases were susceptible to all drugs; 16 and 20 of them were susceptible to STR and INH; none of them were resistant to RIF and EMB respectively. Five (21.74%) and 1 (4.35%) were mono-resistant to STR and INH respectively and 2 (8.7%) were poly-resistant to STR and INH.

Cured cases: The isolates from 16 (39.04%) of the 41 cured cases were susceptible to all drugs; 21 (51.22%), 33 (80.49%), 38 (92.68%) and 39 (95.12%) of them were susceptible to STR, INH, RIF and ETH respectively. Fourteen (34.15%) were resistant or intermediately mono resistant to STR; 2 (4.88%) mono resistant to INH; 2 (4.88%) mono-resistant to RIF; 1 (2.44%) mono resistant to EMB; and 5 (12.19%) poly-resistant to STR and INH.

Defaulted cases: Six (75%) of the defaulted cases were susceptible to all tested drugs; 1 (12.25%) each mono-resistant to STR and poly-resistant to STR and INH respectively.

Dead cases: There were 4 dead cases, 2 (50%) of them were pan-resistant and the remaining 2 (50%) were susceptible to all tested drugs.

Failed cases: One (50%) of the two cases was mono-resistant to RIF and the other was mono resistant to STR.

Relapsed: One was poly-resistant to STR and INH.

X^2 analysis shows that the sensitivity levels of STR (X^2 = 3.3600; P = 0.3394) and INH (X^2 = 3.4915; P = 0.3219) have no influence on treatment outcome. On the other hand, the sensitivity levels to RIF (X^2 = 17.7553; P = 0.0005) and EMB (X^2 = 13.0074; P = 0.0046) have significant associations with treatment outcome (Table 3).

Discussion

The two main objectives of this study were 1) to determine the *in vitro*-drug susceptibilities of isolates obtained from TB patients and 2) to determine whether the clinical responses correlate with the drug susceptibilities of the isolates from those cases, especially the MDRs. The results of *in vitro* antibiotic susceptibility testing can predict the clinical response to treatment and guide the selection of antibiotics [14]. However, the relationship between DST result and clinical outcome is not always straightforward and depends on other parameters such as host factors including immune status, age and co-morbidity. It is well known that in severe bacterial infections, treatment failure can occur when the infecting organism has displayed *in-vitro* susceptibility to the used antibiotics [7,14,15]. In a study on retreatment cases, it was found

Drugs		Cured	Completed	Defaulted	relapsed Failed/died	Total (%)	P. value
STR	Sensitive	21	16	6	4	46 (59.0)	0.3394
	Resistance	19	7	2	4	32 (41.0)	
INH	Sensitive	33	20	7	5	64 (82.1)	0.3219
	Resistance	7	3	1	3	14 (17.9)	
RIF	Sensitive	38	23	8	4	73 (93.6)	0.0005
	Resistance	2	0	0	4	5 (6.4)	
EB	Sensitive	39	23	8	5	75 (96.2)	0.0046
	Resistance	1	0	0	3	3 (3.8)	

Table 3: The drug susceptibility of the various tested drugs was correlated to the treatment outcomes and the findings underscore the importance of rifampicin in TB treatment.

that cavitary disease *per se*, irrespective of drug-resistance status of the *M. tuberculosis* isolate, was associated with poor treatment outcomes [16]. On the other hand certain individuals are able to clear infections with resistance phenotypes; this may be due to the interaction of many factors, among which acquired immunity is presumably important [15]. The correlation between DST result and clinical outcome of antibiotic treatment of several mycobacterial illnesses have been documented in some countries [16,17], however to the authors knowledge, this is the first study conducted in Ghana. Analysis confirmed that resistance to RIF and EMB as well as MDR is predictive of poor response to treatment and this draws attention to the need for a prompt response. There is therefore the need for the National Tuberculosis Control Program to establish in-country commercial rapid DNA-based kits like the Genotype MTBDR-plus (Hain Lifescience, Germany) for testing sputum samples from patients that do not convert to sputum negative by the third month [9]. The MTBDR-plus is a line probe assay that can detect resistance to RIF and INH, in a day and is one of the rapid assays recommended by the World Health Organisation. Interestingly, we did not find INH resistance as important in determining the outcome of treatment in our study populations. At least two molecular mechanisms are known to be involved in INH resistance in *Mycobacterium spp*. The commonest of these are associated with mutations in the *katG* gene which encodes the catalase peroxidase, needed for activation of INH and mutations in the promoter region of the *inhA* gene encoding, NADH-dependent enoyl acyl carrier protein reductase, the primary target for this drug. While mutations in the *katG* gene lead to high levels of INH resistance, mutations in *inhA* promoter generally result in low level resistance [18]. We hypothesised that most of the INH resistant isolates had low-level resistance and therefore the drug could be active *in vivo*, especially given that treatment was by combination therapy.

Findings from this study indicated that about 31%, 15%, 7% and 3% of tested isolates were resistant to STR, INH, RIF and EMB respectively. Mono-resistance and poly-resistance were observed in about a quarter and a tenth of the isolates tested respectively. The primary MDR-TB rate among the study population was 0.9%, MDR among those previously treated cases was 16.67% and the combined was 2.5%, which is higher than the national average of 1.9% [1] as recorded, but comparable to a recent report of 2.2% [19]. Re-treated patients yielded more drug-resistant *M. tuberculosis*, including MDR ($P < 0.001$), than new cases. The level of resistance as observed in our study is intermediate between the two reports on drug resistant TB in Ghana, while that reported in 1989 [20] is on the high side, that published in 2006 [19] has some comparable figures and some lower than what we are reporting. These differences could be the result of regional variations and the commitment of regional TB programs. The isolates used in this study were from cases residing in four main health facilities including health facilities that report stigmatization by both communities and health officials [21]. The need for more education and training on DOTS programs in the regions involved in this study is very essential. Nevertheless, the consensus of the three studies is the high rate of resistance observed to STR and INH, thus making Ghana among countries in Africa with a high rate of resistance [19]. What is more surprising from these studies is the high rate of INH resistance compared to RIF resistance; Ghana as a country has not used INH mono therapy for latent TB, nevertheless the high rate of access to various antibiotics could account for these observed high rate. These findings should serve as a clarion call to action by governments and health officials to deal with the high prevalence of drug-resistant tuberculosis. In addition the national TB program needs to look at abuse of drugs used for TB in treating other infectious diseases. There is also the need to improve treatment compliance and early reporting. The mean duration of seeking healthcare and the high bacterial load as observed in the patients (data not shown) involved in the study suggests a probable high rate of transmission of resistant strains in the community. We are in the process of looking at the transmission of resistant genotypes in the community.

More males reported with TB than females from all the health facilities studies; all together 67% of the participants are males and this compares very well with other reports. The reasons why more men report with TB than women cannot be explained and further investigation on the health seeking behavior of Ghanaian TB patients needs to be done. It could be that women do not have time or they do not have the final decision when to seek help or males engage in more risk activities, hence more prone to TB disease. We are of the opinion that the first reason may be true as shown in table 2, more females reported with difficult to treat TB than males ($p<0.0001$).

Conclusion

We found a high rate of drug resistance among the isolates we analyzed and that treatment outcome depends primarily on the susceptibility of the *M. tuberculosis* isolate to RIF. This confirms the central role of RIF in TB treatment. A conscious effort must therefore be made by the health system to restrict its use in the community. We also found that even though less females report with TB, more females had drug resistant TB compared to males ($p<0.0001$).

Competing Interest

The author(s) declare that they have no competing interests.'

Authors Contribution

Dorothy Yeboah-Manu was involved in the concept and proposal development, design of laboratory analysis and wrote the first draft of the manuscript.

Frank Bonsu was involved in the concept and proposal development and critically reviewed the manuscript.

Adwoa Asante-Poku, Kobina Ampah, Grace Kpeli, Emelia Danso and Kwaku Owusu-Darko were the research assistants. They collected samples, followed cases during treatment, performed all the laboratory analysis.

Acknowledgement

The authors will like to acknowledge the head and staff of the Bacteriology department of Noguchi Memorial Institute for Medical Research, the health personnel and all participants involved in the study. Dr Collins Ahorlu is also acknowledged for statistical analysis and Dr Janet Fyfe of Victorian Infectious Disease Research Laboratory for critical comments on the manuscript. This investigation received financial support from the UNICEF/UNDP/World Bank/WHO special program for research and training in Tropical Diseases (TDR) for Dorothy Yeboah-Manu and the National tuberculosis program, Ghana.

References

1. World Health Organization: Global tuberculosis control: Surveillance, Planning, Financing: Report. Geneva; 2008.
2. Rodger AJ, Toole M, Lalnuntluangi B, Muana V, Deutschmann P (2002) DOTS-based tuberculosis treatment and control during civil conflict and an HIV epidemic, Churachandpur District, India. Bull World Health Organ 80: 451-456.
3. Ormerod L.P. (2005) Multidrug-resistant tuberculosis (MDR-TB): epidemiology, prevention and treatment. Bri Med Bull 73-74: 17-24.
4. World Health Organization Tuberculosis report 2006
5. World Health Organization: Global report on Multidrug and extensively drug-resistant TB (M/XDR-TB). Geneva; 2010
6. Canetti G, Fox W, Khomenko A, Maler HT, Menon NK, et al. (1969) Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull World Health Organ 41: 21-43.
7. Kim S.J (2005) Drug-susceptibility testing in tuberculosis: methods and reliability of results. Eur Respir J 25: 564-569.
8. Piersimoni C, Olivieri A, Benacchio L, Scarparo C (2006) Current perspectives on drug susceptibility testing of Mycobacterium tuberculosis complex: the automated nonradiometric systems. J Clin Microbiol 44: 20-28.
9. Miotto P, Saleri N, Dembelé M, Ouedraogo M, Badoum G, et al. (2009) Molecular detection of rifampin and isoniazid resistance to guide chronic TB patient management in Burkina Faso. BMC Infect Dis 9: 142.
10. Muvunyi C.M., Masaisa F, Bayingana C, Musemakweri A, Mutesa L, et al. (2010) Prevalence and diagnostic aspects of sputum smear positive tuberculosis cases at a tertiary care institution in Rwanda. Afri J Microbiol Res 4: 088-091.
11. Manissero D, Hollo V, Huitric E, Ködmön C, Amato-Gauci A (2010) Analysis of tuberculosis treatment outcomes in the European Union and European Economic Area: efforts needed towards optimal case management and control. Euro Surveill 15: pii: 19514.
12. Asiimwe BB, Koivula T, Källenius G, Huard RC, Ghebremichael S, et al. (2008) Mycobacterium tuberculosis Uganda genotype is the predominant cause of TB in Kampala, Uganda. Int J Tuberc Lung Dis 12: 386-391.
13. Yeboah-Manu D, Yates MD, Wilson SM (2001) Application of a simple multiplex PCR to aid in the routine work of the mycobacterium reference laboratory. J Clin Microbiol 39: 4166-4168.
14. Smaill F (2000) Antibiotic susceptibility and resistance testing: an overview. Can J Gastroenterol 14: 871-875.
15. Böttger EC (2001) Drug-resistant tuberculosis. Lancet 357: 1288-1289.
16. Kritski AL, Rodrigues de Jesus LS, Andrade MK, Werneck-Barroso E, Vieira MA, et al. (1997) Retreatment tuberculosis cases: factors associated with drug resistance and adverse outcomes. Chest 111: 1162-1167.
17. Yew WW, Chan CK, Chau CH, Tam CM, Leung CC, et al. (2000) Outcomes of patients with multidrug-resistant pulmonary tuberculosis treated with ofloxacin/levofloxacin-containing regimens. Chest 117: 744-751.
18. Somoskovi A, Parsons LM, Salfinger M (2001) The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in Mycobacterium tuberculosis. Respir Res 2: 164-168.
19. Owusu-Dabo E, Adjei O, Meyer CG, Horstmann RD, Enimil A, et al. (2006) Mycobacterium tuberculosis drug resistance, Ghana. Emerg Infect Dis 12: 1171-1172.
20. van der Werf TS, Groothuis DG, van Klingeren B (1989) High initial drug resistance in pulmonary tuberculosis in Ghana. Tubercle 70: 249-255.
21. Dodor EA, Kelly S, Neal K (2009) Health professionals as stigmatisers of tuberculosis: insights from community members and patients with TB in an urban district in Ghana. Psychol Health Med 14: 301-310.