Original Article

Bacterial etiology of sexually transmitted infections at a STI clinic in Ghana; use of multiplex real time PCR

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SUMMARY

Background: Most sexually transmitted infection (STI) management efforts focus on the syndromic approach to diagnose and treat patients. However, most women with STIs have been shown to be entirely asymptomatic, or if symptoms exist, are often missed when either clinical or conventional bacteriologic diagnostic tools are employed.
Methods: We assessed the performance of a multiplex real time PCR assay to describe other potential pathogens that could be missed by conventional bacteriological techniques in 200 women attending a routine STI clinic in Kumasi, Ghana.
Results: Although a total 78.00% of the women were asymptomatic, 77.1% of them tested positive for at least one bacterial STI pathogen. *Mycoplasma genitalium* was the most commonly detectable pathogen present in 67.5% of all women. Of those testing positive, 25.0% had single infections, while 38.0% and 19.5% had double and triple infections respectively. Altogether, 86.54% and 90.91% of the symptomatic and asymptomatic women respectively tested positive for at least one pathogen ($p<0.05$). There were no significant associations ($p<0.05$) between the clinical manifestations of the symptomatic women and the pathogens detected in their samples.
Conclusions: Our study confirmed the importance of complementing the syndromic approach to STI management with pathogen detection and most importantly recognise that STIs in women are asymptomatic and regular empirical testing even for both symptomatic and asymptomatic patients is critical for complete clinical treatment.

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INTRODUCTION

Sexually transmitted infections (STIs) are a group of communicable diseases that are transferred predominantly by sexual contact. It is estimated that more than 340 million new cases of curable STIs occur every year throughout the world among adults aged 15-49 years, with the second largest proportion in sub-Saharan Africa.[1] In most developing countries, STIs and their complications are among the top five diseases for which sexually active adults seek health care.[2] Women of childbearing age bear the brunt of major complications resulting from STIs such as infertility, chronic pelvic pain, pelvic inflammatory diseases and ectopic pregnancy.[3]

Several bacterial, viral and fungal pathogens are known to spread by sexual contact. The significant bacterial pathogens include *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) and *Mycoplasma genitalium* (MG). Infections caused by most of these pathogens are known to be curable by employing appropriate antimicrobial treatment regimens.[2]

Although several STI control efforts exist, these usually target symptomatic patients (often men), and have failed to identify asymptomatic individuals (usually women) until serious complications develop.

The World Health Organization (WHO) recommends the syndromic management approach to diagnose and treat persons with STIs. This is based on the identification of consistent groups of symptoms and easily recognizable signs i.e. syndromes, and the provision of treat-

ment targeting the organisms suspected of being responsible for the syndrome. This approach includes counseling and detailed sexual history taking, recording of socio-demographic characteristics, and clinical signs and symptoms.[4] Even though this approach is pragmatic, it is unable to detect infections among asymptomatic or pauci-asymptomatic individuals. Research has shown that most women with STIs are entirely asymptomatic, pauci-symptomatic, or even if symptoms exist, they are often unrecognized.[5,6,7,8] As such many women do not seek timely treatment for STIs, leading to severe underreporting and a gross underestimation of the true incidence and etiology of STIs in women.

Standard diagnostic protocols for STIs such as wet mount and culture exist, but these can be complex, time-consuming and not reliable.[9,11] Due to the potential complications of bacterial STIs and most importantly their interaction with HIV/AIDS,[12] timely and appropriate treatment is crucial for improving health outcomes. Accurate, reliable and timely diagnostic tests for STIs are therefore extremely crucial for both discovering and managing asymptomatic infections. Thus modern molecular diagnostic methodologies which provide a uniform platform for detecting both single and multiple pathogens simultaneously can be very useful in addressing STIs.[13,14] Several studies have underscored the superiority of multiplex real time PCR in pathogen detection in terms of its sensitivity, specificity, reliability, timeliness as well as its advantages over the syndromic approach in STI case detection and management.[13,15,16,17]

In Ghana, relatively few epidemiological surveys employing molecular-based diagnostic techniques have been carried out on the prevalence and etiology of STIs. Studies have largely concentrated on social factors[18,19,20,21,22] predisposing individuals to STIs or have been limited to a number of etiological pathogens.[23,24,25,26] Therefore very little is known about the contribution of other potential bacterial pathogens, particularly intracellular organisms to STIs. This study utilized a multiplex real time polymerase chain reaction (RT-PCR) tool to determine the etiology and magnitude of seven bacterial STI pathogens among women visiting a STI clinic in Kumasi, Ghana.

METHODS

Study setting, population and design

We conducted a cross sectional study at the Suntreso Government Hospital, the main district hospital of the Bantama sub-metropolitan area in the Kumasi metropolis. On yearly basis, this hospital sees approximately 11,700 patients of which 48.72% are STI-related. The hospital runs a dedicated STI clinic which serves as a Specialist and referral centre for treatment and follow up within the Kumasi metropolis. The clinic occasionally performs laboratory tests to diagnose gonorrhea and syphilis using Gram staining and syphilis check rapid tests respectively.

Our study involved women reporting for care at the STI clinic between February and April 2014. Patients were recruited irrespective of whether or not they were symptomatic or asymptomatic. Symptomatic subjects are described as those presenting with any of the following conditions: lower abdominal pain, burning sensation during urination, abnormal vaginal discharge, and abnormal vaginal bleeding. Asymptomatic subjects did not present with any of the symptoms outlined above at the time of visit but had experienced these symptoms in the past. All women reporting to the clinic were eligible for inclusion if they consented to participate.

Data collection and sampling

All patients visiting the clinic within the study period were assigned a number, and 200 of these were selected for inclusion in the study using a computer-generated random process. A standardized questionnaire was administered to each participant by trained STI research assistants. Data on socio-demographic characteristics and current self-reported symptoms including lower abdominal pain, burning sensation during urination, abdominal discharge, and abnormal vaginal bleeding were electronically captured. After general examination by a doctor, an individually wrapped dry cotton flocked swab (Copan, Brescia-Italy) was introduced into the cervical canal of eligible women *per vaginum* by a nurse, and gently rotated without undue discomfort to the patient and withdrawn. The swabs were labeled, placed immediately on ice packs and transported to the laboratories of the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR).

Laboratory processes

DNA extraction

On arrival, we suspended the swabs in 0.5 ml of 1XPhospate-Buffered Saline (PBS) and vortexed them for 30 seconds. The swabs were thereafter discarded and the suspension centrifuged at 10,000xg for 15 minutes at room temperature. The supernatant was discarded and the pellet re-suspended in 190 µl of 1XPBS. Ten microliters of STI-7 internal control (Seegene, Korea) was added and DNA extracted following the Qiagen DNA mini kit protocol (Qiagen GmbH, Hilden, Germany).

Multiplex real time Polymerase Chain Reaction (RT-PCR)

Multiplex RT-PCR was conducted using the Anyplex™ II STI-7 assay (Seegene, Korea) following the manufacturer's instructions. This assay relies on a newly devel-

oped Tagging Oligonucleotide Cleavage and Extension (TOCE™) technology and permits the simultaneous detection of seven nucleic acid targets of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU), *Ureaplasma parvum* (UP) and *Trichomonas vaginalis* (TV). In brief, 5 µl of extracted DNA was added to a PCR tube of Anyplex™ II STI-7 detection reagents, consisting of 5 µl of deionised water, 5 µl of primer, and probe mixture for the seven STI pathogens, and 5 µl of Anyplex™ II PCR master mix. We then performed the DNA amplification and detection on a CFX96™ Biorad real-time PCR platform (Biorad, Singapore) associated with the Seegene viewer software (Seegene, South Korea) per the manufacturer's guidelines. Positive and negative controls were included per run. Results were given as positive or negative.

Ethics, consent and approval

We obtained ethical approval from the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology. We also sought permission from the Head of the STI clinic of our study hospital. We explained the aims and objectives of our study to potential participants, after which verbal consent was obtained from those willing to take part in the study. Written informed consent was also secured, expressed by the participants appending their signatures or thumbprint to the informed consent form.

Data Management and Statistical Analysis

Data were electronically captured with Android Smart Phone technology using the CommCare application software (CommCare ODK, version 2.18), sent through the cloud which aggregated into a Microsoft Excel file in a central computer. Data were cleaned and then exported into Stata (version 12.0; Stata Corp LP, College Station, TX, USA) for analysis. Descriptive statistics were summarized and displayed in tables, graphs and charts. For continuous variables, estimates were for the means, with their standard deviations, while discrete variables were analysed using chi-square tests. We calculated the overall prevalence of the microorganisms in the population and as well, for mono, double and multiple infections. Logistic regression analysis was performed on binary data, and 95% confidence levels assuming a default alpha of 5%.

RESULTS

Characteristics of the study population

The average age of the women was 29.3±8.4 years (range 11-68). More than half of the study population (53.50%) were between 21-30 years old, and 40% were married. Forty percent (40%) of the population had at least a primary level of education, 88.0% were Christians and 79.50% were Asante speaking Akans (Table 1).

Table 1 Characteristics of the study population

Variable	Category	Frequency (%)
Age group (years)		
	<20	19 (9.50)
	21 – 30	107 (53.50)
	31 – 40	54 (27.00)
	41 +	20 (10.00)
Marital status		
	Married	79 (39.50)
	Living together	44 (22.00)
	Divorced	6 (3.00)
	Widowed	4 (2.00)
	Single	67 (33.50)
Education level		
	Illiterate	18 (9.00)
	Primary	80 (40.00)
	Secondary	65 (32.50)
	Tertiary	37 (18.50)
Occupation		
	Student	33 (16.5)
	Salaried worker	28 (14.0)
	Trader	38 (19.0)
	Unemployed	37 (18.5)
	Others	64 (32.0)
Religion		
	Christian	176 (88.0)
	Muslim	23 (11.50)
	No religion	1 (0.50)
Ethnicity		
	Akan	159 (79.90)
	Ga/Dangme	2 (1.01)
	Ewe	1 (0.05)
	Northerner	10 (5.00)
	Other groups	28 (14.57)

Aetiology of STIs and co-infections

Overall, 87.5% of the 200 women screened had at least one positive STI pathogen. Of those testing positive, 25.0% had one positive pathogen (single infections), while 38.0% and 19.5% were positive for two and three

pathogens respectively and 5.0% had four pathogens per sample.

Of the pathogens detected, MH was the most common, present in 67.5% of the women, UP in 62.5%, and UU in 33.5%. TV was the least detectable pathogen and was present in only one individual.

Association between Clinical presentation and pathogen detection

A total 78.00% of the women were symptomatic. They were younger (age range, 14-57 years; mean, SD, 28.56±7.7 years) compared to the 44 asymptomatic patients (age range, 11-68 years; mean, SD, 32.05±10.2 years).

The commonest presenting complaint of symptomatic women was abnormal vaginal discharge; 68.5%, followed by 31% experiencing burning sensation during urination, 29% with pelvic pain, and 9% with abnormal vaginal bleeding. A total of 86.54% of the symptomatic participants tested positive for at least one pathogen compared with 91% of the asymptomatic patients who had at least one detectable pathogen in their sample (χ^2=0.59, p=0.439). Of the symptomatic women, 78.52% tested positive for CT, compared with 21.48% of the asymptomatic women (χ^2=0.07, p=0.799). A further 76.8% and 29.2% of the symptomatic and asymptomatic women respectively were positive for UP (χ^2=0.28, p=0.597). See Table 2.

Table 2 Pathogen detection in symptomatic and asymptomatic women

Pathogen (N)	Symptomatic N (%) (N= 156)	Asymptomatic N (%) (N=44)	χ^2 (p-value)
MH (135)	106 (78.52)	29 (21.48)	0.07 (0.799)
UP (125)	96 (76.80)	29 (29.20)	0.28 (0.597)
UU (67)	56 (83.58)	11 (16.42)	1.83 (0.176)
NG (15)	13 (86.67)	2 (13.33)	0.71 (0.400)
MG (9)	5 (55.56)	4 (44.44)	2.77 (0.096)
CT (7)	4 (57.14)	3 (42.86)	1.84 (0.175)
TV (1)	1 (100.00)	-	-

Our results revealed no significant associations (p<0.05) between the clinical manifestations as presented by the symptomatic women and the pathogens detected in their samples.

Patient age and pathogens

Of the 175 women who tested positive for a pathogen, the highest detections (94/175) were observed in those within the 21-30 year bracket followed by those within the 31-40 (49/175) and 40+ (17/175) groups. The group with the least detections was the less than 11 (15/175). There was no association between patient age and the pathogens detected (p<0.05) (Table 3).

Table 3 Distribution of patients according to age group and detected pathogen.

Age *	CT (%)	NG (%)	MH (%)	MG (%)	UU (%)	UP (%)	TV (%)
<20	1 (5.26)	1 (5.26)	13 (68.42)	1 (5.26)	6 (31.58)	11 (57.89)	
21-30	4 (3.74)	6 (5.61)	72 (67.29)	6 (5.61)	37 (34.58)	61 (57.01)	1 (0.93)
31-40	1 (1.85)	6 (11.11)	40 (74.07)	2 (3.70)	18 (33.33)	39 (72.22)	
>40	1 (5.00)	2 (10.00)	10 (50.00)	0	6 (30.00)	14 (70.00)	
χ^2	0.76	1.88	3.865	1.353	0.198	4.206	0.874
pt-value	0.859	0.597	0.276	0.717	0.978	0.24	0.832

* Age category in years, pt-value: p-value for trend

DISCUSSION

This study reports on the use of a multiplex real time PCR system to determine bacterial etiology and distribution of STI pathogens among women visiting a STI clinic in Ghana. Our detection rate of 87.5% compares to that found in France (70.4%) where the same detection methodology (Anyplex II STI-7) was employed.[14] Our prevalence rate is however higher than those in previous studies where rates of 1.9-17% have been observed in five cities in West Africa.[27] Particularly, our rate is higher compared with other studies in Ghana which found rates ranging from 0.6-7.7%.[24,28] However there could be some bias in comparing our study with some previous studies in the sub region. These may include the different study population such as our case where our site is a major referral STI hospital, the detection methodologies employed and most importantly the number of pathogens targeted. Most studies have relied on sex workers[26] and pregnant women attending antenatal and gynecological clinics.[28,29]

Additionally, all these studies focused predominantly on two key bacteria pathogens (Chlamydia and gonoccocal infections).[28,29] Furthermore, even though these studies employed nucleic acid detection assays, they focused on single detections. Except for special circumstances, such as antimicrobial sensitivity testing, nucleic acid detection assays have become the tests of choice because they can rapidly detect multiple pathogens simultaneously via their multiplex function. Consequently, multiplex PCR assays obviates individual detections, allows faster and reliable detections, and reduces labour and reagent costs as well.[17]

At most health facilities, multiplex real time PCR is not the recommended approach towards case management due to its associated cost implications especially in resource poor countries. Using this multiplex detection system, our study identified single, multiple and as well co-infections in the study population that otherwise would not have been detected. This indicates that unlike the syndromic approach to STI case detection management where treatment is based on signs and symptoms, the multiple pathogen detection approach allows for the detection of the most important sexually transmitted pathogens, thereby providing a means for a more thorough evaluation of the clinical significance of the various organisms. Epidemiological surveys using this approach could be advantageous since they can be used to provide information on the bacterial agents most frequently responsible for STIs in women and better inform the syndromic case management approach.

Our PCR methodology revealed high detection rates for MH and the two *Ureaplasma spp* (UU and UP) in both symptomatic and asymptomatic women. This conforms to previous studies in the USA where these pathogens were detected in 40-80% and 21-53% of symptomatic and asymptomatic women respectively.[30] The role of these organisms as STI pathogens needs to be empirically determined due to the controversy surrounding them. For example, even though UP has been associated with fatal pregnancy outcomes,[31] its role as a STI pathogen remains unclear. Further investigations into the pathogenicity of these organisms is necessary.

Our total detection rates of 3.5% for CT and 7.5% for NG is also consistent with other studies in Ghana where rate ranges of 3.0 -7.7 and 0.6-3.4% for CT and NG respectively have been reported.[24,26,28,29,32] However, when comparing these pathogens among symptomatic and asymptomatic women, CT was frequently detectable in the latter. This further strengthens the need to complement the syndromic approach with pathogen detection in the management of STIs especially for women who tend to be entirely asymptomatic or pauci-symptomatic.

Unlike a previous study in Ghana which reported 6.7% of TV detections among women attending a STD clinic, our data suggested a low detection in the population. Generally, TV is known to have a low prevalence.[14,16] It is therefore possible that due to its generally low prevalence among women, our nucleic acid detection assay may have a low positive predictive value for this pathogen as it has already been reported.[17] However, it will be highly relevant to conduct further studies to determine its relevance in STIs especially in women.

In this study, the chief complaint of the women was abnormal vaginal discharge. The reason for this could be because our study population comprised of women. It is known that almost all STI etiological agents, be they bacterial, protozoan, viral or fungal have manifestations of vaginal discharge in women.[33] Higher rates of similar syndromes have also been reported in Ethiopia.[34]

The absence of significant associations between the pathogens detected and self-reported symptoms has implications for the syndromic approach to STI case management. Even though this approach avoids expensive laboratory tests, can easily be implemented at the primary health care level and is particularly sensitive among symptomatic people, its numerous associated disadvantages outweighs some advantages. Key among the disadvantages is its inability to detect infections among asymptomatic or pauci-symptomatic women. Our study detected pathogens in more that 90% of the asymptomatic women. As shown by several studies, since many STI infected women are entirely asymptomatic or pauci-symptomatic,[32,35,36] it is needful to adopt a pathogen detection approach of key STI organisms in case management. This will prevent over diagnosis and over treatment which also portend an increase in drug resistance, changes in vaginal flora and possible side effects of multiple drugs.

CONCLUSION

Our study confirmed the importance of increasing vigilance against STIs by complementing the syndromic approach with specific pathogen detection, and most importantly recognizing that STIs in women are often pauci-symptomatic or entirely asymptomatic and regular empirical testing for them is crucial.

The multiplex real time PCR methodology employed proved useful in identifying seven STI pathogens in our study population. The generally high STI pathogen detection rates, especially in our asymptomatic study participants, further confirms the relevance of exhaustive testing using sensitive methods to prevent unnecessary

treatment with antibiotics. Our study further contributes to our understanding of the epidemiology of bacterial etiologic agents of STIs in symptomatic and asymptomatic women in Ghana and makes a case for further attention to be paid to addressing case management strategies to diminish the burden of the disease.

Our study was limited in a number of ways. Firstly, we did not have access to data on other key factors such as behaviour, biology and social factors with the potential to influence the acquisition of the bacterial pathogens of STIs. Further studies should therefore be conducted to stratify according to sociological, biological and behavioural background. In addition, future studies may involve other viral and fungal pathogens of STI to enable a holistic approach towards STI case management. Although multiplex real time PCR has a disadvantage of being expensive and requires highly skilled personnel to deliver, some level of emphasis should be placed on it at least in referral hospitals and research institutions to help improve STI case management.

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REFERENCES

1. Duru C, Emele F, Nnebue C, Adinma E, Ifeadike G, Amilo G, et al. The Seroprevalence and Co-Existence of Chlamydia Trachomatis and Herpes Simplex Virus Antibodies among Students in a Tertiary Institution. *International Journal of Medical Sciences*. 2014;1(8):122-30.
2. Organization WH. Global prevalence and incidence of selected curable sexually transmitted infections: overview and estimates. 2001.
3. Organization WH. Global incidence and prevalence of selected curable sexually transmitted infections—2008. WHO, Geneva, Switzerland. 2012.
4. Organization WH. Guidelines for the management of sexually transmitted infections: World Health Organization; 2003.
5. Adler M. Sexually transmitted diseases control in developing countries. *Genitourinary Medicine*. 1996;72:83 - 8.
6. Chippindale S, Lau R, Radcliffe K. STIs at the millennium. Past, present, and future. Report on the conference held 3-7 May 2000, Baltimore. *Sexually Transmitted Infections*. 2000;76:218 - 9. PubMed PMID: doi:10.1136/sti.76.3.218.
7. Peterman T, Tian L, Metcalf C, Satterwhite C, Malotte C, DeAugustine N, et al. High incidence of New sexually transmitted infections in the year following a sexually transmitted infection, a case for rescreening. *Annals of Internal Medicine*. 2006;145:564 - 72.
8. Lewis D, Latif A, Ndowa F. WHO global strategy for the prevention and control of sexually transmitted infections, time for action. *Sex Transmitted Infections*. 2007;83:508 - 9. PubMed PMID: doi:10.1136/sti.2007.028142.
9. Swygard H, Seña AC, Hobbs MM, Cohen MS. Trichomoniasis: clinical manifestations, diagnosis and management. Sexually Transmitted Infections. 2004 April 1, 2004;80(2):91-5.
10. Watson EJ, Templeton A, Russell I, Paavonen J, Mardh P-A, Stary A, et al. The accuracy and efficacy of screening tests for Chlamydia trachomatis: a systematic review. Journal of Medical Microbiology. 2002;51(12):1021-31.
11. Van Dyck E, Ieven M, Pattyn S, Van Damme L, Laga M. Detection of Chlamydia trachomatis and-Neisseria gonorrhoeae by Enzyme Immunoassay, Culture, and Three Nucleic Acid Amplification Tests. *Journal of clinical microbiology*. 2001;39(5):1751-6.
12. Boily M-C, Alary M, Baggaley RF. Neglected issues and hypotheses regarding the impact of sexual concurrency on HIV and sexually transmitted infections. *AIDS and Behavior*. 2012;16(2):304-11.
13. Screm M, Santolo MD, Scarparo C, Arzese A. P2.047 Evaluation of a Multiplex Real-Time PCR Assay For Rapid Detection of *C.trachomatis* and *N. Gonorrhoeae* from Genital Clinical Specimens. *Sexually Transmitted Infections*. 2013 July 1, 2013;89(Suppl 1):A102.
14. Berçot B, Amarsy R, Goubard A, Aparicio C, Loeung HU, Segouin C, et al. Assessment of co-infection of Sexually Transmitted pathogen microbes by performing the molecular kit Anyplex™ II STI-7. *Journal of Clinical Microbiology*. 2014 December 24, 2014.
15. Pettiforn A, Walsh J, Wilkins V, Raghunathan P. How effective is syndromic management of STDs?: a review of current studies. *Sexually Transmitted Diseases*. 2000;27:371 - 85. PubMed PMID: doi:10.1097/00007435-200008000-00002.
16. Moon SJ, Choi JE, Park KI. Comparison of the Anyplex II STI-7 and Seeplex STD6 ACE Detection Kits for the Detection of Sexually Transmitted Infections. *Journal of Laboratory Medicine and Quality Assurance*. 2013;35(2):87-92.
17. Choe H-S, Lee DS, Lee S-J, Hong S-H, Park DC, Lee M-K, et al. Performance of Anyplex™ II multiplex real-time PCR for the diagnosis of seven sexually transmitted infections: comparison with currently available methods. *International Journal of Infectious Diseases*.17(12):e1134-e40.

18. Appiah-Agyekum NN, Kayi EA. Students' perceptions of contraceptives in University of Ghana. *Journal of Family and Reproductive Health*. 2013 Mar;7(1):39-44. PubMed PMID: 24971101. Pubmed Central PMCID: PMC4064744. Epub 2013/03/01. eng.
19. Bankole A, Biddlecom A, Guiella G, Singh S, Zulu E. Sexual behavior, knowledge and information sources of very young adolescents in four sub-Saharan African countries. *African Journal of Reproductive Health*. 2007 Dec;11(3):28-43. PubMed PMID: 18458739. Pubmed Central PMCID: PMC2367131. Epub 2008/05/07. eng.
20. Rondini S, Krugu JK. Knowledge, attitude and practices study on reproductive health among secondary school students in Bolgatanga, upper east region, Ghana. African *Journal of Reproductive Health*. 2009 Dec;13(4):51-66. PubMed PMID: 20690273. Epub 2010/08/10. eng.
21. Biddlecom AE, Munthali A, Singh S, Woog V. Adolescents' views of and preferences for sexual and reproductive health services in Burkina Faso, Ghana, Malawi and Uganda. *African Journal of Reproductive Health*. 2007 Dec;11(3):99-110. PubMed PMID: 18458737. Pubmed Central PMCID: PMC2367115. Epub 2008/05/07. eng.
22. Asampong E, Osafo J, Bingenheimer JB, Ahiadeke C. Adolescents and parents' perceptions of best time for sex and sexual communications from two communities in the Eastern and Volta Regions of Ghana: implications for HIV and AIDS education. BMC *International Health and Human Rights*. 2013;13:40. PubMed PMID: 24070548. Pubmed Central PMCID: PMC3849363. Epub 2013/09/28. eng.
23. Apea-Kubi KA, Yamaguchi S, Sakyi B, Kishimoto T, Ofori-Adjei D, Hagiwara T. Neisseria gonorrhoea, Chlamydia trachomatis, and Treponema pallidum infection in antenatal and gynecological patients at Korle-Bu Teaching Hospital, Ghana. Japanese *Journal of Infectious Diseases*. 2004 Dec;57(6):253-6. PubMed PMID: 15623949. Epub 2004/12/30. eng.
24. Opoku B, Sarkodie Y. Prevalence of genital Chlamydia and Gonococcal infections in at risk women in the Kumasi metropolis, Ghana. Ghana Medical Journal. 2010;44(1).
25. Agyarko-Poku T, Sarkodie YA, Frimpong EH. P3.232 Prevalence of Mycoplasma Genitalium Among Women Attending Sexually Transmitted Infection Clinic in Kumasi, Ghana. *Sexually Transmitted Infections*. 2013 July 1, 2013;89(Suppl 1):A221.
26. Agyarko-Poku T. P3-S7.18 Aetiological agents of infective vaginal discharge among women attending a STD clinic in Kumasi, Ghana. *Sexually Transmitted Infections*. 2011 July 1, 2011;87(Suppl 1):A305.
27. Pepin J, Deslandes S, Khonde N, Kintin D, Diakité S, Sylla M, et al. Low prevalence of cervical infections in women with vaginal discharge in west Africa: implications for syndromic management. *Sexually transmitted infections*. 2004;80(3):230-5.
28. Bentsi C, Klufio C, Perine P, Bell T, Cles L, Koester C, et al. Genital infections with Chlamydia trachomatis and Neisseria gonorrhoeae in Ghanaian women. *Genitourinary Medicine*. 1985;61(1):48 - 50.
29. Apea-Kubi K, Shinya Y, Sakyi B, Kisimoto T, Ofori- Adjei D, Hagiwara T. Neisseria gonorrhoeae, chlamydial trachomatis and treponema pallidum infection in antenatal and gynaecological patients at Korle-Bu Teaching Hospital Ghana. *Japanese Journal of Infectious Diseases*. 2004;57:253 - 25616.
30. Waites KB, Katz B, Schelonka RL. Mycoplasmas and ureaplasmas as neonatal pathogens. *Clinical Microbiology Reviews*. 2005;18(4):757-89.
31. Sung T-J. Ureaplasma infections in pre-term infants: Recent information regarding the role of Ureaplasma species as neonatal pathogens. *Korean Journal of Pediatrics*. 2010;53(12):989-93.
32. Yirenya-Tawiah D, Annang TN, Apea-Kubi KA, Lomo G, Mensah D, Akyeh L, et al. Chlamydia Trachomatis and Neisseria Gonorrhoeae prevalence among women of reproductive age living in urogenital schistosomiasis endemic area in Ghana. *BMC Research Notes*. 2014;7(1):349.
33. Moges B, Yismaw G, Kassu A, Megabiaw B, Alemu S, Amare B, et al. Sexually transmitted infections based on the syndromic approach in Gondar town, northwest Ethiopia: a retrospective study. *BMC Public Health*. 2013;13(1):143. PubMed PMID: doi:10.1186/1471-2458-13-143.
34. Wolday D, G-Mariam Z, Mohammed Z, Meles H, Messele T, Seme W, et al. Risk factors associated with failure of syndromic treatment of sexually transmitted diseases among women seeking primary care in Addis Ababa. *Sexually Transmitted Infections*. 2004;80:392 - 4. PubMed PMID: doi:10.1136/sti.2003.005660.
35. Sloan NL, Winikoff B, Haberland N, Coggins C, Elias C. Screening and Syndromic Approaches to Identify Gonorrhea and Chlamydial Infection among Women. *Studies in Family Planning*. 2000;31(1):55-68.
36. Dallabetta G, Gerbase A, Holmes K. Problems, solutions, and challenges in syndromic management of sexually transmitted diseases. *Sexually Transmitted Infections*. 1998;74:S1 - S11.✪