Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Ilorin, Nigeria

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Summary

Background: **Nosocomial infections caused by methicillin-resistant strains of *Staphylococcus aureus* constitute significant epidemiologic problems. Defining an outbreak requires the use of rapid and highly discriminatory epidemiologic methods to determine the epidemic strains involved in such outbreak.**

Study design: **A descriptive laboratory based surveillance study for MRSA was undertaken. One hundred and forty seven *Staphylococcus aureus* isolates from clinical specimens were screened for methicillin resistance at the University of Ilorin Teaching Hospital between January and December 2001. Fifty one (34.7%) methicillin resistant strains recovered were epidemiologically characterized using *Eco R1* restriction enzyme analysis of their plasmid DNAs.**

Result: **Forty five (88.2%) MRSA isolates were associated with infections and 6 (11.8%) were colonizing strains; 36 (70.6%) and 15 (29.4%) were hospital and community acquired respectively. Skin and soft tissues were sites of infection in 36 (70.6%) cases and surgical, emergency and ICU accounted for 33 (64.7%) isolates. All isolates were resistant to more than two antibiotics but sensitive to vancomycin. Forty two (82.4%) isolates contained plasmids including 9 (21.4%) that contained more than one plasmid. Restriction Enzyme Analysis of the Plasmid DNA (REAP) divided the isolates into 9 *Eco R1* profiles, with profile 2 accounting for 41.7% of all nosocomial infections in the wards, implying that it is endemic. The remaining nosocomial profiles occurred less frequently, suggesting that they are sporadic strains originating from outside the hospital. The community strains showed diverse digestion pattern indicating that they are from different clones.**

Conclusion: **The spread of MRSA can be controlled through reinforcement of appropriate use of antibiotics, hand washing and laboratory surveillance for MRSA, particularly in the surgical wards and intensive care units, in order to identify sources of outbreaks.**

Key-words: ***Molecular, Methicillin-Resistant, Staphylococcus aureus***

Résumé

Introduction: Infections nosocomiales provoquées par résistant au méthicilline tension d'aureuse staphylococcus constitue des problèmes épidemiologiques importants. Afin de définir un declenchement demande l'utilisation d'une méthode épidemiologique fortement discriminatiore et rapide pour évaluer les tensions épidemiques impliquées dans un tel déclenchement

Plan d'étude: Une étude descriptive de surveillance laboratoire de base pour MRSA a été effectuée cent quarante sept isolates aureus staphylococcus de spécimens cliniques ont été selectionés pour le résistant au méthicilline au centre hospitalier universitaire d'Ilorin entre janvier et décembre 2001. Cinquante et un soit 34,7% tension de la résistance méthicilline retrouvées ont été épidemiologiquement classées avec l'utilisation d'analyse de la restriction d'enzyme Eco R de leur plasmid DNAS.

Résultats: Quarante cinq soit 88,2% MRSA isolates ont été liés avec infections et 6 soit 11,8% étaient des tensions de colonisation: 36 soit 70,6% et 15 soit 29,4% étaient acquis dans l'hôpital et dans la communauté respectivement. La peau et les parties charnues étaient les sièges de l'infection en 36 soit 70,6% des cas, et chirurgical; urgence et ICU constituent 33 soit 64,7% isolates, Tous les ilolates étaient rebelles au plus de deux antibiotiques mais très sinsible au vaneomycine, quarante deux soit 82,4% isolates avaient plasmids y compris 9 soit 21,4% qui ont plus d'un plasmid. L'analyse de la Restriction d'Enzyme de DNA plasmid (REAP) a divisé les isolates en 9 Eco RI profiles, avec le profile 2 qui constitue 41,7% de toutes des infections nosocomiales dans les salles, ce qu'implique qu'il est endémique. Les autres profiles nosocomials étaient recensés comme moins fréquemment; ce qui suggère qu'ils sont des tensions sporadiques d'origine d'exterieur de l'hôpital. Les tensions communautaires ont indiqué la tendance de digestion diverses ce qu'implique qu'ils sont d'origines de clones divers.

Conclusion: On pourrait controller la propagation de MRSA à travers le renforcement de l'utilisation des antibiotiques nécessaires: se laver la main, et la surveillance laboratoire pour MRSA, surtout dans la salle

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Table 4 *Eco RI* **plasmid DNA digestion profiles**

Profile (P)	Molecular Weight of fragment band (kbp)	No of isolates (%)	Nosocomial (%)	Community acquired (%)
P0	No plasmid	9 (17.6)	3 (8.3)	6 (40)
P1	2.32, 4.36, 23.13	7 (13.7)	6 (16.7)	1 (6.7)
P2	2.32, 23.13	15 (29.4)	15 (41.5)	0
P3	4.36, 21.23, 23.13	4 (7.8)	3 (8.3)	1 (6.7)
P4	2.32, 4.36, 9.42, 23.13	8 (15.7)	8 (22.2)	0
P5	9.43, 21.23	3 (5.9)	1 (2.8)	2 (13.3)
P6	2.03, 21.23	2 (3.9)	0	2 (13.3)
P7	23.13	1 (1.9)	0	1 (6.7)
P8	2.32	2 (3.9)	0	2 (13.3)
		51	**36**	**15**

**Undigested plasmid*

Table 5 Plasmid DNA content of MRSA isolates

Number of plasmid	Molecular weight range	Number of isolates
0	–	9
1	9.42 – 23.13	33
2	21.23, 23.13	8
3	9.42, 21.23,23.13	1
>3	–	0
		51

typeable[5, 21]. Lyon and co-workers[7] and Zuccarelli and his colleagues[6] have demonstrated the diversity and stability of restriction enzyme profiles of plasmid DNA from MRSA. They found very few of MRSA strains obtained from different sources and locations to lack plasmids entirely and assures the wide applicability of profiling to MRSA epidemiology, superior to phage typing and surface protein electrophoresis.

Table 6 Ward distribution of *Eco RI* **profiles of nosocomial isolates**

Profile/Ward	Surgical		Medical	ICU	Emergency	Total
	W5	W2				
Profile 0	1	2	0	0	0	3
Profile 1	2	3	0	0	1	6
Profile 2	5	4	1	4	1	15
Profile 3	2	1	0	0	0	3
Profile 4	4	0	2	2	0	8
Profile 5	0	0	0	1	0	1
Profile 6	0	0	0	0	0	0
Profile 7	0	0	0	0	0	0
Profile 8	0	0	0	0	0	0
	14	**10**	**3**	**7**	**2**	**36**

Discussion

The MRSA prevalence rate of 34.7% in this study compares favourably with the prevalence reported in Turkey, France, Italy, and Spain[14, 15] but less than that reported in other parts of Nigeria[16, 17], Senegal[18], Latin America[19] and Japan[20].

In characterizing MRSA isolates epidemiologically, antibiotic profile is poorly discriminatory, type specific antisera for serologic tests are expensive, phage typing are often non-reproducible and some strains are non-

Restriction Enzyme Analysis of Plasmid DNA (REAP) resolved all the MRSA isolates in this study into 9 *Eco RI* profiles. Profile 2 occurred in all the units and accounted for 41.5% of the nosocomial infections indicating that this profile is endemic in this hospital and may be responsible for outbreak, which has probably passed unnoticed. Four of the seven isolates in the ICU including the three that had similar antibiotic profile, four of the isolates in orthopaedic ward and five of the isolates in general surgical ward belonged to profile 2. There is

also clustering of MRSA infections between June and September (See Bar Chart) during which profile 2 strains accounted for about 50% of the infection.

Profile 2 strain may be an "epidemic strain" that has spread from an index case to other patients. In this institution, patients are frequently being moved from ICU to the wards and personnel from one ward to the other. We were not able to screen hospital staff for carriage of MRSA in order to detect source of this strain, as there is no concrete infection control programme in this institution.

Profiles 0, 1, 3, 4 and 5 accounted for the remaining nosocomial infections and were not found in all the wards while profiles 6, 7, and 8 were not found among the nosocomial isolates. Profiles 1 and 3 though occurred less frequently, may be subtypes of profile 2 as they differ by only one plasmid fragment band. Other profiles are infrequently isolated and this suggests sporadic occurrence of these strains in this hospital.

The community acquired strains also had different profiles from each other and only 4 of the 15 community isolates shared profiles 0, 1, 3 and 5 with the nosocomial isolates. The different digestion pattern also suggests sporadic occurrence in the community.

In this study, 9 of 51 (17.7%) MRSA isolates did not contain detectable plasmid (8.3% nosocomial isolates and 40% community isolates). This is at variance with the findings of Zuccarelli and his colleagues[6] who reported only 4.5% in their study but agrees with Hartstein and coworkers[5] who reported 33% in their study. Those isolates without plasmids were categorized profile 0 and were therefore not characterized by this epidemiologic method. A more precise method of identification would have been a combination of two or more PCR based methods such as *mec A* gene PCR, RAPD, Coagulase gene PCR, 16S-23S rRNA spacer amplification technique with pulse field gel electrophoresis of endonuclease digest of these amplified chromosomal DNA[22]. Unfortunately, these methods are beyond the reach of most clinical laboratories in Africa.

Conclusion

The prevalence of MRSA in this institution is relatively high with MRSA isolate having REAP DNA profile 2 being the most prevalent type. Routine hand washing by hospital personnel, use of safe and aseptic techniques as well as policy regulating antibiotic prescription and usage will reduce nosocomial infection in this environment. Though REAP DNA profile is less discriminatory as an epidemiologic tool than the polymerase chain reaction (PCR) based methods, it is much easier to isolate and purify plasmid DNA than the chromosomal DNA and also relatively cheaper to perform plasmid extraction than PCR in our environment.

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Chronic hepatitis in Nigerian patients: a study of 70 biopsy-proven cases

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Summary

Background: **Liver cirrhosis and hepatocellular carcinoma are known sequelae of chronic hepatitis. Early diagnosis and treatment of chronic hepatitis could delay or even abort progression to terminal liver disease.**
Study design: **Prospective study of 70 consecutive patients with features of early liver disease or discovered with HBsAg (or anti-HCV) during pre-employment and/or pre-donation screening at Ile-Ife, Nigeria. All the patients had liver biopsy and the histology evaluated with the Knodell Histological Activity Index.**
Result: **Fifty-three patients had symptomatic disease (M: F ratio, 1.5:1) while 17 were asymptomatic (M: F ratio, 3:1). The mean ages were 49.04 (SD±16.78) and 29.82 (SD±6.13) for the symptomatic and the asymptomatic patients respectively (P< 0.005). Major symptoms were right upper abdominal pain (68%), weight loss (51%) and fatigue (41.5%). Alcohol consumption was significantly related to symptomatic chronic hepatitis (P< 0.01). Over 50% of patients with asymptomatic chronic hepatitis had abnormal liver scan and liver function tests. All the asymptomatic cases and 77.4% of the symptomatic group had HBsAg while only 1 patient (symptomatic) was anti-HCV positive. On liver histology, all the patients with asymptomatic chronic hepatitis had a Knodell score of ≤8 and none had fibrosis. Over half of the symptomatic patients had a Knodell score of ≥ 9 (56.6%) and stage 2 or 3 fibrosis (51%).**
Conclusion: **Asymptomatic chronic hepatitis patients tend to be younger and of the male sex. Symptomatic chronic hepatitis may signal the onset of significant fibrosis and alcohol abuse may accelerate this process. Serum ALT and liver scan are useful initial screening tests for asymptomatic patients with hepatitis B or C viral markers.**

Key-words: ***Chronic hepatitis, Asymptomatic, Symptomatic, Hepatitis B, Hepatitis C, Histology, Knodell score.***

Résumé

Introduction: Cirrhose du cullin foie, maladie du foie et le carcinome hépathocellulaire sont connus comme la séquelle de l'hépatite chronique. Un diagnostic précoce et traitement d'hépetite chronique pourrait retarder ou bien terminer la progression jusqu'au terminal de la maladie du foie.
Plan d'étude: Etude en perspective de 70 patients consecutifs avec les traits de la maladie du foie précoce ou découvert avec HbsAg (ou anti-HCV) pendant pré-emploie et/ou selection prédomination à Ile-Ife, Nigeria. Tous les patients avaient la biopsie du foie et on avait évalué l'histologie avec l'indice d'activité histologique de knodell.
Résultats: Cinquante trois patients étaient atteints de la maladie symptomatique (de proportion M: F 1, 5:1) tandis que 17 étaient asymptomatiques d'une proportion (M:F, 3:1). Les ages moyens étaient 49,04 (SD ± 16, 78) et 29; 82 (SD±6,13) pour des patients symptomatiques et asymptomatiques respectivement (P<0,005). Les symptoms principaux sont la douleur abdominale du côté droit superieur (68%), perte du poid (51%) et faiblesse (41,5%) consomption d'alcool était sensiblement liée au symptomatique hépatite chronique (P<0,01). Plus de 50% des patients avec l'asymtomatique hépatite chronique avaient subi l'examen de scan du foie anormal et fonction du foie. Tous les cas d'asymptomatiques et 77,4% du groupe de symptomatique avaient HbsAg tandis que un patient (symptomatique) seulement était anti-VCH positif. A travers l'histologie du foie, tous les patients atteints d'asymptomatique d'hépatité chronique avaient un score de knodell de ≤ et aucun cas de la fibrose. Plus d'un demi des patients symptomatiques avaient eu le score de ≥ 9 (56,6%) et 2me ou 3me étape de la fibrose (51%).
Conclusion: Les patients asymptomatiques avec hépatite chronique pourrait indiquer l'importance de la fibrose du début et excès d'alcool pourrait augmenter ce processus. Sérum ALT et scan du foie sont des selections de début très utile pour l'asymtomatique avec l'hépatite B ou marquers C viral.

Introduction

Chronic hepatitis has been defined as continuing inflammation of the liver without improvement for at least six months [1]. Aetiological agents include hepatitis B, C and D, drugs, autoimmune and genetic disorders. It is a recognised precursor lesion for cirrhosis, the final irreversible stage of chronic hepatitis, and ultimately, hepatocellular carcinoma (HCC). Unfortunately, the symptoms of chronic hepatitis are often non-specific and

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definitive diagnosis depends on histological confirmation. Hepatitis B virus (HBV)-associated liver disease is a common condition in Nigeria as in many other sub-Saharan African countries [2]. Most patients with HCC in this region are positive for HBV markers and do present with a short history of symptoms [3,4]. Diagnosis of chronic liver disease at a very early stage is therefore crucial if chances for cure are to be enhanced, a prospect now made brighter by the advent of specific chemotherapy of chronic viral hepatitis [5].

In spite of the high prevalence of HBV-associated chronic liver disease in Nigeria, there is hardly any study in the country examining the clinico-pathological characteristics of chronic viral hepatitis. It is hoped that the information obtained from such a study may help in the early detection and treatment of chronic hepatitis so that progression to irreversible liver disease or malignancy can be averted.

Patients and methods

This was a prospective study of consecutive patients seen at the University Teaching Hospital (a tertiary health care centre) at Ile-Ife, Nigeria between 1987 and 2002. Patients with symptoms suggestive of liver disease and persons who either tested positive to viral hepatitis markers in their blood or had abnormal liver function tests during routine medical/blood donation screening were selected for investigation. As much as possible, patients with overt features of decompensated liver disease were excluded. Those selected were investigated with real time ultrasonography and liver biopsy after appropriate history and physical examination. Their serum biochemistry and haematology profile were also done. Screening for hepatitis B surface antigen (HBsAg) in the blood was done with Latex Kit, BIOTEC Laboratories Ltd., UK while ELISA test (2nd generation) was employed for the detection of antibodies to hepatitis C virus (anti-HCV). All asymptomatic subjects seropositive for either viral marker had a repeat serology after 6 months in order to eliminate cases of acute hepatitis. Regular liver ultrasound scan and serology for viral markers were only commenced in our hospital years into this study with anti-HCV test being the most recent (introduced only 3 years to the end of study). Therefore, not all the subjects selected could undergo these investigations. Screening for HBeAg and HBV-DNA, markers of HBV replicative activity, were not done due to lack of facilities.

Liver biopsy assessment

To obtain biopsies of the liver, the one-second Menghini technique[6] was adopted, using the trans-thoracic approach after securing informed consent. The liver tissue sample obtained averaged 1.84cm (SD ± 0.52) in length. In each case, the liver tissue specimen was fixed in buffered 10% formaldehyde, processed routinely within 24 hours and embedded in paraffin wax. Six 3 μm sections were cut from each block and subjected to the following stains: haematoxylin and eosin, reticulin (Gordon and Sweet) Masson's trichrome (for fibrosis assessment), Perl's Prussian blue (for iron), periodic acid Schiff (PAS) with diastase digestion (to check for α-1-antitrypsin) and the Shikata's orcein stain (for HBsAg). Only those subjects with histological features compatible with chronic hepatitis were included in the study. In all cases, two pathologists examined the stained tissue sections at different sessions. The histological grade (degree of necroinflammatory activity) and stage (degree of fibrosis) were scored using the Knodell Histological Activity Index (HAI)[7]. Grades of necroinflammation were grouped according to the proposal of Desmet *et al*[8], with slight modification.

Statistical analysis

Tests of statistical analysis were done using the Student's t-test and the Chi-Square test as appropriate. P value of < 0.05 was regarded as significant.

Results

A total of 70 patients, 45 males and 25 females (M:F = 1.8:1) were studied. Their ages ranged from 20 to 78 years with a mean of 44.37 (SD ± 17.02). Fifty-three (75.7%) of the 70 chronic hepatitis patients were symptomatic while 17 (24.3%) were asymptomatic. The symptomatic patients were made up of 32 males and 21 females with a mean age of 49.04 years (SD ± 16.78). The asymptomatic group consisted of 13 males and 4 females (M:F = 3:1) with an age range of 20 to 44 years and a mean age of 29.82 (SD ± 6.13) ($P < 0.005$). The asymptomatic subjects were detected at the time of attempted blood donation (n = 10) and during routine medical tests (n = 7). The most common symptom among the symptomatic group was right upper quadrant abdominal pain (68%) followed by weight loss (51%) and body fatigue (41.5%) (Table 1). History of jaundice in the past was positive in only 26.7% of the cases while 86.5% had no history of blood transfusion.

Significant alcohol consumption defined as ≥ 60g/day for males and ≥ 40g/day for females for not less than 10 years was found in only 18 patients. Of these 18 cases,

Table 1 Main symptoms among chronic hepatitis patients (n = 53)

Symptoms	No.(%)
Abdominal pain (right upper)	36 (68)
Weight loss	27 (51)
Body fatigue/weakness	22 (41.5)
Jaundice	18 (34)
Abdominal swelling	16 (30.2)
Fever	15 (28.3)
Anorexia	14 (26.4)
Leg oedema	14 (26.4)
Early satiety	10 (18.9)
Diarrhoea	10 (18.9)

Table 2 Serum *ALT, liver ultrasound scan and viral hepatitis markers in chronic hepatitis patients

	Serum *ALT			Liver Ultrasound Scan			HBsAg					Anti-HCV				
	Normal	Abnormal	Total	Normal	Abnormal	Total	Neg.		Pos.		Total	Neg.		Pos.		Total
							M	F	M	F		M	F	M	F	
Asymptomatic	7	8	15	7	8	15	–	–	13	4	17	7	4	-	-	11
Symptomatic	9	26	35	5	18	23	2	5	19	5	31#	2	3	1	-	6
Total	16	34	50	12	26	38	2	5	32	9	48	9	7	1	-	17

**ALT = Alanine transaminase #For 6 of these patients the HBsAg was positive only in the liver tissue*

however, 15 (83.3%) belonged to the symptomatic group ($P < 0.01$). Serum alanine transaminase (ALT) could only be done in 50 patients (35 symptomatic and 15 asymptomatic) as reagents were occasionally out of stock. Thirty-four (68%) of them had abnormal serum ALT levels while they were normal in 16 (32%). The 34 patients consisted of 26 symptomatic (74.3%) and 8 asymptomatic patients (53.3%). Seventy-eight percent of the symptomatic and 53.3% of the asymptomatic patients had abnormal liver scan. All of the asymptomatic patients were HBsAg positive but all of the 11 of them tested for anti-HCV were negative. Of the 31 symptomatic patients tested for HBsAg, 18 (58%) were positive. However, HBsAg was stained in the liver tissue of 6 additional patients bringing the total HBsAg positivity rate among them to 77.4%. Ten out of the 21 female symptomatic patients had HBsAg screening done out of whom 5 (50%) were positive. Anti-HCV screening could only be done in 7 of all the 25 female patients and all were negative. Only 1 of the 6 symptomatic patients tested was positive for anti-HCV (see Table 2).

Of the 18 patients with significant alcohol consumption, 10 were screened for HBsAg and of these, 7 (70%) were positive. None of the asymptomatic patients had hepatomegaly or splenomegaly. On the other hand, 42 (79.2%) and 18 (34%) of the symptomatic patients had hepatomegaly and splenomegaly respectively.

Correlation of histological findings with clinical features

The histological evaluation of the liver tissues from all the 70 patients was based on the histological activity index (HAI) of Knodell or Knodell score. All of the asymptomatic patients had a histological grade of ≤ 8 (maximum score = 18) with 11 (64.7%) of them below grade 5 (Table 3). None of the members of the asymptomatic group had fibrosis on histology (stage 0). On the contrary, 30 (56.6%) of the 53 symptomatic patients had a histological grade of ≥ 9. Eleven (20.8%) of them scored a grade of ≥ 14 demonstrating severe necroinflammation and early cirrhosis (stage 4). All the patients with histological stage 3 or 4 were symptomatic. Features of liver cell dysplasia (LCD) were seen in 4 cases (1 asymptomatic, 3 symptomatic). All the 4 cases (3 males and 1 female) with LCD were positive for HBsAg and had a histological grade of ≤ 9. The female patient returned 3 years after the diagnosis of chronic hepatitis was made with evidence of neoplastic transformation as shown by increased hepatomegaly and the development of hepatic arterial bruit.

Discussion

Chronic hepatitis can present with or without symptoms. Even when it is symptomatic, the symptoms can be non-specific[8]. This study confirms this observation and it also shows that symptomatic chronic hepatitis is significantly related to age ($P < 0.005$), as has been reported elsewhere[9]. Hepatitis B virus (HBV) infection is usually acquired in childhood in sub-Saharan Africa. If the mean period of exposure to HCV to the development of cirrhosis (i.e. 21 years)[10] is applicable to HBV infection, clinical disease would therefore be expected to begin to manifest by middle age. For the same reason, asymptomatic patients are younger and, as also shown by this study, predominantly males. The male sex is a recognised risk factor for chronic HBV carrier state[11].

Alcohol consumption was also shown by this study to be significantly associated with the development of symptomatic chronic hepatitis ($P < 0.01$). Symptomless HBsAg carriers drinking ethanol have been shown to be

Table 3 Correlation of clinical status of chronic hepatitis with liver histological grading and staging scores

*HAI	Grading Score (over 18)						Staging Score (over 4)				
Clinical Status	3–4	5–6	7–8	9–13	≥14	Total	0	1	3	4	Total
Asymptomatic	11	4	2	–	–	17	14	3	–	–	17
Symptomatic	3	16	4	19	11	53	19	7	16	11	53
Total	14	20	6	19	11	70	33	10	16	11	70

**Histological Activity Index*