



FOREWORD

An ideal HIV vaccine requires the development of neutralizing antibodies that are able to halt infection by viral strains from across the world. The recent isolation of twelve monoclonal antibodies that are highly effective against HIV subtype C and their potential as a new tool for HIV prevention, particularly in South Africa, is reviewed in this issue. Plans for hepatitis B surveillance in South Africa are also reviewed. Hepatitis B infection prevalence in South Africa is high with rural populations most affected. This issue also includes a review of the potential of the anti-tuberculosis agent pyrazinamide for the management of drug-resistant tuberculosis infections.

Surveillance reports include hepatitis A which, in South Africa, is characterised by localised and widespread institutional and community outbreaks. Laboratory confirmed hepatitis A case statistics for 2011, 2012 and 2013 are given in this issue, which also includes antimicrobial resistance surveillance statistics for the most important disease causing pathogens in South Africa in 2013. Lastly, the results of malaria vector surveillance at Vlakbult, Mpumalanga Province, for the 2012/2013 malaria season are given, and show a comparatively high preponderance of the major malaria vector *Anopheles arabiensis*.

All contributors are thanked for their inputs, and I trust you will find these diverse reports useful and interesting.

Basil Brooke, Editor

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DEVELOPMENT OF BROAD NEUTRALIZING ANTIBODIES TO HIV – IMPLICATIONS FOR VACCINE DESIGN

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A future preventative HIV vaccine is likely to require the development of neutralizing antibodies that are able to halt infection by viral strains from across the world. These types of neutralizing antibodies are termed “broadly neutralizing antibodies (bNAbs)”. Although no vaccine tested thus far has been able to elicit bNAbs, studies over the last decade have shown that approximately 20% of infected people naturally develop these kinds of antibodies following several years of infection. Although this is of no clinical benefit to the people who produce them (as this only occurs after HIV infection is well-established), the identification of these rare people has allowed the HIV vaccine field to examine precisely how bNAbs develop. The premise of such studies is that defining the pathway to a broad spectrum of activity (neutralization breadth) will enable the design of novel vaccine strategies designed to elicit bNAbs prior to infection.

Clues from studying HIV infection

Since 2003, researchers in the Centre for HIV and STIs have been following a cohort of HIV-infected women who are part of the Centre for the AIDS Programme of Research in South Africa (CAPRISA) cohorts in KwaZulu-Natal province. Of the 40 women who were first enrolled, seven naturally developed bNAbs. One of these individuals (known as CAP256) developed particularly potent bNAbs, and unusually did so within the first year of HIV infection. Detailed studies of her blood antibodies showed that these targeted a particular site of vulnerability on the virus (referred to as V1V2) that is well protected by sugars. bNAbs to V1V2 generally have a very unusual structure, with long “arms” referred to as complementarity determining region H3 (or CDR H3) that are able to penetrate between the sugars that surround V1V2. How such antibodies develop these characteristic long CDR H3s, and become broadly neutralizing, has not previously been described.

NICD/CAPRISA researchers, in collaboration with US researchers at the NIH Vaccine Research Centre (Washington, DC) and Columbia University (New York), this year published in *Nature* the first description of how this class of V1V2-targeting bNAbs developed in CAP256, providing important insights for vaccine design.¹

Potent antibodies isolated from a HIV-infected South African woman

Twelve somatically related neutralizing antibodies (CAP256-VRC26.01–12) were isolated from donor CAP256 by B cell culture. These antibodies were tested against viruses from across the world, and were able to neutralize between 15% and 46% of all global viruses (figure 1), but showed better neutralization of South African subtype C viruses (66% breadth). Mapping studies showed these antibodies recognized the V1V2 epitope that is a common target of bNAbs. All twelve antibodies had the long CDR H3 characteristic of bNAbs to V1V2, which protruded high above the rest of the antibody structure.

Deep sequencing of the antibody genes in CAP256 showed that the CAP256-VRC26 family of antibodies developed between 30 and 38 weeks post-infection. By examining the earliest sequences of related antibodies, the un-mutated common ancestor for the family was inferred and characterized. This ancestor antibody, like the mature antibodies, had a fully formed long CDR H3 loop which was created by VDJ recombination, and not through a gradual process of affinity maturation. This initial antibody was already capable of strain-specific neutralization. Breadth, however, was shown to require a moderate level of somatic hypermutation (the introduction of mutations into the antibody genes, resulting in increased potency and breadth). This moderate level of somatic hypermutation is in contrast with the extremely high levels of mutations required for breadth by many other bNAbs.

How will this advance HIV prevention efforts?

The elucidation of the developmental pathway of this class of bNAbs has significant implications for HIV vaccine design. Future immunogens will be required to engage naïve B cells bearing antibodies with pre-formed long CDR H3 “arms”. However, these types of antibodies are very rare in humans, as they are frequently autoreactive, and are therefore deleted by the immune system. The engagement of these rare cells will need novel approaches to vaccine design. On the other hand, once these rare naïve B cells are activated, they require much less affinity maturation than other bNAbs, and can therefore become broadly neutralizing within weeks or months, rather than years – potentially good news for HIV vaccine design.

In the meantime, the isolation of twelve monoclonal antibodies that are highly effective against the subtype C HIV strains that dominate the epidemic in sub-Saharan Africa provide valuable new tools for HIV prevention. The availability of these antibodies, which can be expressed at high levels in the laboratory, presents exciting opportunities for their passive administration into people at risk of becoming infected, or those already infected. Recent studies in non-human primates have shown that such passive transfusions of broadly neutralizing antibodies can protect from infection, and reduce viral loads. These antibodies, likely in combinations with other mAbs targeting conserved epitopes, are therefore a valuable addition to our arsenal of anti-HIV monoclonal antibodies, and will progress into non-human primate studies in the coming months.

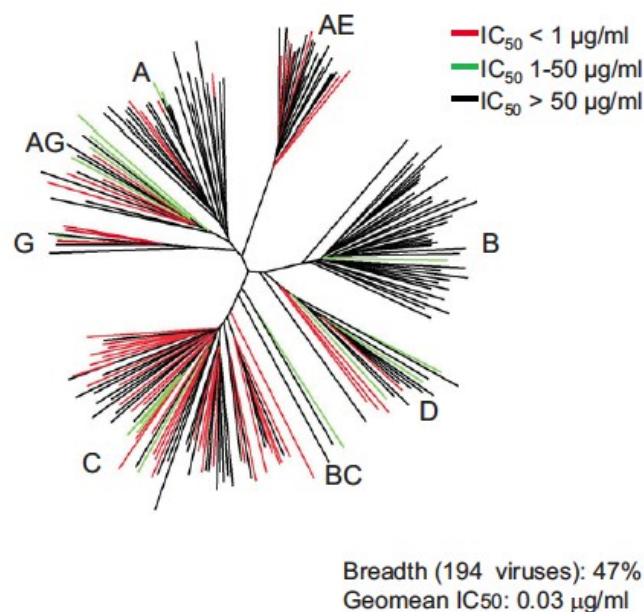


Figure 1: Neutralization breadth and potency of CAP256-VRC26.08, the broadest of this family of antibodies, against 194 viruses from multiple subtypes is shown. Viruses are arranged in a phylogenetic tree, and coloured red if neutralized potently (<1 µg/ml), green for less potent neutralization (1-50 µg/ml) and black if resistant to neutralization. CAP256-VRC26.08 is most effective against subtype C viruses, which are the predominant subtype in South Africa.

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THE LANDSCAPE FOR HBV-RELATED SURVEILLANCE IN SOUTH AFRICA: WHERE ARE WE NOW?

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Introduction

Hepatitis B virus (HBV) is a major global public health problem, with 2 billion people infected and 360 million carriers.¹ Individuals with chronic HBV have a high risk of developing liver cirrhosis and liver cancer.² The World Health Organization estimates that 15% to 25% of adults who become chronically infected during childhood will die from HBV-related liver cancer or cirrhosis.³ Geographic prevalence of hepatitis B can be classified as low (<2%), intermediate (2-7%) or high (>8%).⁴ Previously published studies have estimated a high prevalence of greater than 10% in South Africa.^{5,6}

Vaccination against HBV has been available to infants in South Africa since 1995 as part of the Expanded Programme on Immunisation (EPI).⁷ The vaccine is routinely administered at 6, 10 and 14 weeks of age, with some private schedules including a booster at 18 months.⁸ A birth dose is not currently part of the EPI schedule, although it has recently been recommended by the World Health Organisation.⁴ There have been no catch-up vaccinations for adolescents or school-based vaccination programmes. There are limited reports on assessing the effectiveness of the vaccine⁷ and national estimates of vaccine coverage range from 73% to 96%.⁹ There is no mandatory requirement for vaccination of risk groups such as healthcare workers.

In South Africa, HBV is primarily transmitted horizontally before the age of 5 years. There are higher prevalence rates in rural areas than urban areas¹⁰, which is attributed to poor hygiene and greater chances of HBV transmission through cuts, insect bites, use of contaminated needles, tribal scarification and ear piercing using contaminated equipment.¹¹ Sexual transmission is thought to account for the majority of infections in adolescents and adults. Occupational risk groups, such as health care workers, are at risk from

HBV transmission from parenteral and percutaneous exposures. HIV and HBV virus co-infection is common^{12,13}, and HIV is known to accelerate the progression of chronic HBV infection to end-stage disease.¹⁴

Owing to the natural history of hepatitis B disease, South Africa is likely to continue to experience the effects of HBV infection despite almost two decades of universal Hepatitis B vaccination. Robust and timely information on HBV infection and disease would therefore provide valuable evidence to inform the planning and evaluation of public health interventions in South Africa, including infant HBV vaccination, targeted vaccination of adolescents, the need for screening or vaccination among risk groups such as healthcare workers, and the provision of timely treatment for those already infected. However, there is currently no national system for ongoing, routine assessment of the burden of HBV infection and disease in South Africa.

In January 2014, the National Institute for Communicable Diseases (NICD) hosted a one-day expert workshop to explore existing and potential opportunities for developing HBV-related surveillance in South Africa. In this commentary a summary of these opportunities is presented and discussed.

Notified cases via the District Health Information System

HBV is a notifiable disease in South Africa, and notifications are collated within the District Health Information System (DHIS). However, these data are subject to incomplete case ascertainment for HBV, and as such are likely to underestimate the number of diagnosed cases of HBV. Alternative mechanisms to quantify the burden of infection are therefore required.

The NHLS Corporate Data Warehouse (CDW)

All tests performed through National Health Laboratory Service (NHLS) laboratories in South Africa are compiled in the Corporate Data Warehouse (CDW). Information on tests and test results for HBV serological markers are included in this dataset. The CDW therefore provides a wealth of data on HBV infection among those tested for HBV. These data could be used to investigate the distribution of tested and diagnosed infections across the country, by gender and age group, which in turn could inform an understanding of the distribution of disease and pathways into care for those infected.

Analyses using CDW data are subject to some important limitations which should be considered. Firstly, results are limited to samples which have been tested for HBV markers. As the majority of tests have been carried out for diagnostic purposes, these data do not represent the general population. Thus, the numbers of diagnoses represent rates of infection among those tested, rather than incidence of infection in the general population. Secondly, the CDW includes repeated samples taken from the same person, but unique identifiers for each individual tested within the dataset are not available. This means that the number of HBV diagnoses will be overestimated where repeat testing has occurred. Furthermore, repeat testing limits the possibility of correctly determining the stage of an infection if previous test results are not linked to a current infection. In order to address this limitation, combinations of name, gender and date of birth can be used to indicate likely matches between samples. Algorithms for use of CDW data for HBV monitoring are currently being developed.

The South African Blood Service (SANBS)

All potential blood donors in South Africa are screened for HBV and other blood borne viruses. Those who test positive for HBV are informed of their results and referred for further management. As blood donors are a self-selecting population who are at lower risk of HBV than the general population, data on the proportion of blood donors infected can provide an indication of the lower bounds of HBV prevalence in the general population.

Seroprevalence in the general population

Given the limitations of the CDW and SANBS data, further understanding of the undiagnosed prevalence of HBV infection and HBV immunity would be required to provide a more complete picture of the burden of HBV and the effectiveness of current control measures in South Africa.

HBV seroprevalence information from a general population sample could be used to improve an understanding of the undiagnosed prevalence of infection, thus indicating potential unmet treatment needs or likelihood of future disease. Seroprevalence data among antenatal populations can provide an indication of undiagnosed prevalence, as well as inform discussions around HBV screening in pregnancy.^{15,16} Incorporating testing for HBV serological markers into existing repeated cross-sectional, population-based health surveys, or of existing unlinked anonymous surveys of antenatal clinic attendees, might also prove informative and therefore warrants further consideration.

HBV infection among high risk groups

There are limited opportunities at present for monitoring the burden of infection among specific at-risk groups such as healthcare workers, men who have sex with men (MSM), commercial sex workers, HIV positive individuals, infants born to HIV positive mothers, patients undergoing dialysis, or injecting drug users. The contribution of these groups to ongoing transmission of infections, and possible interventions aimed at one or more of these groups, also requires attention.

Monitoring HBV vaccination

One of the primary potential benefits of HBV-related surveillance would be to inform the monitoring and evaluation of HBV vaccination in South Africa. The EPI compiles data on HBV vaccination coverage, using routinely collected data from the DHIS. These data could be usefully supplemented using biological markers to indicate levels of vaccine-induced immunity among those born since 1995 (i.e. those born since the introduction of HBV vaccination as part of the EPI). Serological testing has been used to demonstrate the success of HBV vaccination.^{17,18} However, these studies have been based on specific cohorts (usually in health

care facilities, for easily accessible sampling) and in specific locations in South Africa. National surveillance of school-aged children could be undertaken to indicate numbers of children that missed vaccinations. Such surveillance could supplement existing data, and could be used to deliver a catch-up adolescent programme.⁷

The NICD is exploring future opportunities to obtain serum samples from young adults for HBV seroprevalence testing using cross-sectional surveys, and by using residual blood samples from other monitoring systems targeted at this age group.

Monitoring HBV-related disease

Surveillance of HBV-related morbidity and mortality will provide valuable evidence to demonstrate the scale of the public health problem caused by HBV, and provide an outcome measure to demonstrate the effectiveness of prevention and treatment activities. However, surveillance of HBV disease from routine clinical data will be subject to substantial challenges, as HBV is not the only cause of liver disease or liver cancer, and incomplete case ascertainment is likely as not all cases will access care.

The National Cancer Registry (NCR) offers one source of HBV-related disease data. Since 1995, the NCR has

collected information on all cases of histologically or cytologically diagnosed hepatocellular carcinoma. Hepatocellular carcinoma cases are currently grouped with cancers of the liver or bile duct, but separate reporting may be possible.

Conclusion

The prolonged natural history of hepatitis B infection and disease creates challenges for surveillance, leading to varied case definitions and tools in use in various countries.^{19,20} Several data sources exist which could usefully contribute to surveillance of HBV infection, immunity and disease in South Africa. The limitations of these data sources mean that several approaches are required, and are currently being explored as the framework for a national surveillance report.

Acknowledgements

The authors thank Dr. Monique Andersson, Dr. Mark Sonderup, Prof. Wendy Spearman, Dr. Elizabeth Prentice, Mr Muzi Hlanzi, Dr. Marion Vermeulen, Dr. Elvira Singh, Dr. Seymour Williams, Dr. Dumisile Venessa Maseko, Prof. Lucille Blumberg, Prof. Jeffrey Mphahlele, Dr. Chris Hoffman and Dr. Rose Burnett for input into the one day workshop and for their invaluable experience and advice.

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PYRAZINAMIDE SUSCEPTIBILITY TESTING AND IT'S USE IN DRUG SUSCEPTIBLE AND DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* INFECTIONS

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Introduction

Pyrazinamide (PZA) is an important anti-tuberculosis agent and has been used as a first-line therapy in combination with rifampicin, isoniazid and ethambutol for the treatment of drug susceptible tuberculosis for many years. With the emergence of drug resistance in *Mycobacterium tuberculosis* (MTB), the causative

pathogen of tuberculosis, much interest has been focussed on the potential of PZA as a second-line agent in shorter treatment regimens.¹⁻³

Mechanism of action and characteristics of pyrazinamide

Pyrazinamide is a pyrazine analogue of nicotinamide

and is hydrolysed intracellularly to pyrazinoic acid (POA) by the enzyme pyrazinamidase (PZase) which is encoded by the *pncA* gene in *M. tuberculosis*. Although the action and exact target of POA is unknown it is believed to disrupt cellular membrane potential leading to cell death. A study by Shi et al¹ identified the ribosomal protein S1 (coded by RpsA) as an additional or novel target of POA by detecting RpsA mutations among PZA resistant strains that do not exhibit resistant *pncA* mutations. This mechanism of inhibiting trans-translation could explain the sterilising ability of PZA by eradicating non-replicating (dormant) mycobacteria.¹

Resistance to pyrazinamide

Mycobacterium tuberculosis develops resistance by means of mutations in the *pncA* gene leading to loss of PZase activity.⁴ Other resistance mechanisms, e.g. inhibition of trans-translation at the ribosomal level which is associated with mutations in the RpsA region, have been described.¹ The presence of PZA resistance can be determined phenotypically and genotypically.

Challenges with phenotypic testing of pyrazinamide

Drug susceptibility testing is complicated by the fact that PZA is only active at low pH levels. Testing on solid media is of limited value as the growth of *M. tuberculosis* is not sustainable at these low pH levels. Liquid systems (Bactec™ MGIT™ 460/960) are preferable by utilizing a modified test protocol which incorporates an acidified culture medium (pH 6). Poor reproducibility and false resistance classifications up to as high as 68% have been reported, and a combination of phenotypic and genotypic methods are now recommended to reach 100% positive predictive value for PZA resistance (phenotypic screening and genotypic confirmation [*pncA* mutations] of all resistance).⁵

Current approaches for phenotypic testing

Several phenotypic testing approaches are available and include conventional inhibition of growth using solid agar at a pH of 5.5, growth inhibition within liquid media using the Bactec MGIT 960 system at a pH of 6.0⁶, pyrazinamidase assays to detect the activity of the enzyme e.g. Wayne method⁷, a colorimetric in vitro synthesized method⁸ and pyrazinoic efflux assays.⁹ These approaches all differ with regard to sensitivity and

specificity.

Current approaches for molecular resistance detection

Owing to the multiple mutation/mutation combinations across the entire *pncA* gene that confer resistance to PZA, amplification and detection of specific mutations is not sufficient. Sequencing across the entire *pncA* gene is recommended for the detection of resistance mutations.¹⁰⁻¹² Although the majority of PZA resistant strains harbour *pncA* mutations, other mutations have been described. Sequencing outside of the *pncA* gene, e.g. in the promoter region, as well as other genes, may be necessary to improve the sensitivity of resistance detection.¹³ Whole genome sequencing offers the advantage of detecting all known as well as new mutations potentially associated with resistance.

Use of PZA in the treatment of drug resistant tuberculosis

Mono- or isolated resistance to pyrazinamide will have little impact on the efficacy of first-line treatment regimens. However, unlike isolated resistance to streptomycin and ethambutol, prolonging the treatment duration of isoniazid and rifampin for a further 3 months is recommended. It is also worth noting that most PZA mono-resistant isolates are likely to belong to *Mycobacterium bovis*, a species which falls within the *M. tuberculosis* complex, and such isolates would not be routinely speciated.¹⁴ Resistance to combinations of pyrazinamide with any other first-line agent would also result in longer treatment duration. PZA should always be included in treatment if tested susceptible in a multi-drug resistant tuberculosis (MDR TB) case. However, due to challenges with susceptibility testing, it is usually included in MDR TB regimens.

Discussion

Despite the challenges of PZA susceptibility testing, efforts for the optimization of this drug should continue as the potential benefit of using it in the management of drug resistant tuberculosis may well be underestimated.

The mechanism of action of PZA on dormant mycobacteria is poorly understood and further research is needed to elucidate this phenomenon and to develop

methods to overcome PZA resistance.

Currently, there is no gold standard for the determination of PZA resistance. Phenotypic testing can be problematic because of reproducibility as well as difficulties with culturing in low pH conditions. The possibility of development of new mutations that could render organisms resistant to PZA also needs to be explored. Although not routinely implemented, a combination of phenotypic screening followed by PCR or whole genome sequencing to confirm the presence of known resistance mutations should be incorporated either routinely or at least in research related to methodologies for PZA resistance determination, in order to ensure accurate results. This, however, will incur an enormous cost.

The use of PZA as part of a multidrug regimen may or may not have advantages. The inclusion of this drug in regimens directed at multi-drug resistant tuberculosis, irrespective of PZA susceptibility status, and the outcome of this practice has not been adequately investigated.

The advent of new anti-tuberculosis drugs such as bedaquiline, sutezolid and delamanid, together with the

promising anti-tuberculosis properties of moxifloxacin, and their incorporation into novel anti-tuberculosis drug regimens for short duration treatment of tuberculosis irrespective of resistance status to current anti-tuberculosis drugs, shows considerable promise. A role for PZA, if any, in these new formulations will have to be determined, as combinations of novel anti-tuberculosis drugs show sterilizing activity.¹⁵

Against a background of increasing drug resistance and decreasing availability of new or novel therapeutic agents, optimization of the use of PZA could be beneficial. This includes standardization of susceptibility testing. Further research is necessary to improve current testing methods, while a continuing search for new clinically relevant mutations linked to PZA resistance remains a priority. Finally, studies are required to evaluate clinical outcomes associated with the inclusion of PZA in MDR TB patient drug regimens.

It is concluded that the usefulness of PZA as a sterilising agent in standard first-line anti-tuberculosis treatment affords it cornerstone status in the treatment of drug susceptible tuberculosis. Its role and optimal use in the management of drug resistant tuberculosis requires further study.

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LABORATORY-CONFIRMED HEPATITIS A IN THE SOUTH AFRICAN PUBLIC HEALTH SECTOR, 2011-2013

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Introduction

Hepatitis A virus (HAV), a non-enveloped positive-stranded RNA picornavirus, is the most common cause of acute viral hepatitis in the world.¹ Hepatitis A is transmitted primarily via the faecal-oral route, but can also be transmitted through ingestion of contaminated food or water, through direct contact with an infectious person (i.e. sexual intercourse), or through injecting drug use. Symptoms usually appear 28 days after exposure, but the incubation period can range from 15 to 50 days.^{2,3}

The expression of hepatitis A infection is variable and includes asymptomatic infection, symptomatic hepatitis with or without jaundice, fulminant hepatitis with acute

liver failure, cholestatic hepatitis, and relapsing hepatitis. The clinical presentation is age-dependent: in children <5 years, most (80 - 95%) infections are asymptomatic, in contrast to adults in whom 70 - 95% of infections are symptomatic.^{1,3,4} Signs and symptoms of hepatitis A infection may include fever, fatigue, loss of appetite, nausea, vomiting, abdominal pain, dark urine, pale stools, arthralgia and jaundice. A humoral immune response to HAV infection begins to occur prior to the onset of symptoms; IgM antibodies to HAV (anti-HAV IgM) are detectable at or prior to onset of clinical illness, and decline to undetectable levels by about 3 - 6 months.⁵

Acute hepatitis A infection is diagnosed through testing anti-HAV IgM. Molecular detection methods (e.g. HAV

PCR on blood and faeces) are not widely available and not routinely used for diagnosis of hepatitis A infection.

In South Africa, localised and widespread institutional and community outbreaks occur, and are often problematic to control.^{6,7} Hepatitis A is a notifiable medical condition and legislation requires that all cases be reported by healthcare workers to the Department of Health through the notification system. In reality this notification system has many challenges (including poor compliance with passive notification by healthcare workers), and as a result data generated are incomplete. In addition, notified hepatitis A cases reflect only those with overtly symptomatic disease. In 2008 and 2009, 342 and 83 cases of hepatitis A were notified to the National Department of Health respectively. In 2009, data reported/notified were from only two of the nine provinces.⁸ The last published HAV serosurvey data for South Africa dates from studies performed in the 1980s and 1990s.¹ The results showed very high seropositivity in black populations, slightly lower rates in mixed race populations and Asian populations, and intermediate rates in white populations.⁹⁻¹⁶ Incidence rates of hepatitis A are strongly related to socioeconomic indicators and access to safe water and adequate sanitation facilities. As income rises and access to safe water increases, the incidence of HAV infection decreases.⁴ Given the spectrum of socioeconomic conditions and differential access to safe water available to South Africans, it is likely that HAV infection incidence rates vary considerably across different communities and regions of the country.

The aim of this study was to provide an estimate of the incidence of HAV disease in the public health sector in South Africa for the period January 2011 to December 2013 using data obtained from the National Health Laboratory Service (NHLS) Corporate Data Warehouse (CDW). NHLS-CDW is a centralised laboratory information system (LIS) from which data on all laboratory tests performed at NHLS laboratories throughout the country can be accessed.

Methods

Tests were conducted at referral NHLS laboratories throughout the country. Hepatitis A infection was

confirmed by a positive serological test for anti-HAV IgM.

Data on all anti-HAV IgM-positive cases for the period 1 January 2011 to 31 December 2013 were extracted from the NHLS-CDW. Data were edited to check for duplicate records, miscoding, missing and out-of-range values. Duplicates were removed and the data verified.

Incidence rates were calculated by dividing the number of laboratory-confirmed cases by the population at risk over a specified time period and are expressed as cases per 100 000 population. Incidence rates were calculated using the mid-year population estimates for 2011 to 2013 from Statistics South Africa.¹⁷ Data from private laboratories were not included in the analysis.

Results

From January 2011 to December 2013, 7 973 laboratory-confirmed cases of hepatitis A were reported through NHLS laboratories. Comparing the number of cases reported through the years, the frequency of cases reported in 2013 was slightly higher than the other years (table 1). However, overall incidence rates were similar in all three years (ranging from 5.0 cases per 100 000 population in 2012 to 5.2 in 2011 and again in 2013). Figure 1 illustrates the number of laboratory-confirmed hepatitis A cases by month and year. Although a higher proportion of cases was reported during the month of May each year, no seasonal variation in disease was noted.

Hepatitis A cases were reported from all nine South African provinces. In terms of geographical distribution, Gauteng (2 073, 26.0%), Western Cape (1 931, 24.2%) and KwaZulu-Natal (1 324, 16.6%) provinces accounted for the highest proportions of the total number of cases reported. The number of cases also differed within provinces with certain districts (in particular, the metropolitan areas) accounting for the highest proportion of total cases reported. Rates of disease incidence differed by province with Western Cape Province recording the highest incidence (table 1). Comparing rates of disease in 2013 to the previous years, a decrease in incidence was observed in the Free State and Gauteng provinces, while an increase was

observed in KwaZulu-Natal Province (table 1).

Age was reported in 92% (7 327/7 973) of the cases. Age ranged from <1 year to 110 years (median age 10 years). Children aged <10 years accounted for a higher proportion of cases (n= 3 472, 47.4%), with 25.0%

(n=1 834) aged <5 years. Overall age-specific incidence ranged from 4.6 cases per 100 000 population in 2012 and 2013 to 4.9 in 2011, but was highest in the <5 year and 5 - 9 year age groups (figure 2). The incidence of disease decreased with increasing age (figure 2).

Table 1: Number of laboratory-confirmed hepatitis A cases in the public health sector and incidence rates by province, South Africa, 2011-2013.

Province	2011		2012		2013	
	n*	Incidence**	n*	Incidence**	n*	Incidence**
Eastern Cape	204	3.0	179	2.7	204	3.1
Free State	166	6.0	153	5.6	75	2.7
Gauteng	777	6.9	680	5.5	617	4.8
KwaZulu-Natal	249	2.3	431	4.2	644	6.2
Limpopo	210	3.8	199	3.6	205	3.7
Mpumalanga	172	4.7	274	6.7	169	4.1
North West	53	1.6	112	3.2	94	2.6
Northern Cape	64	5.8	45	3.9	66	5.7
Western Cape	726	13.7	538	9.1	667	11.1
South Africa	2 621	5.2	2 611	5.0	2 741	5.2

* number of hepatitis A cases; **Incidence rates are expressed as cases per 100 000 population

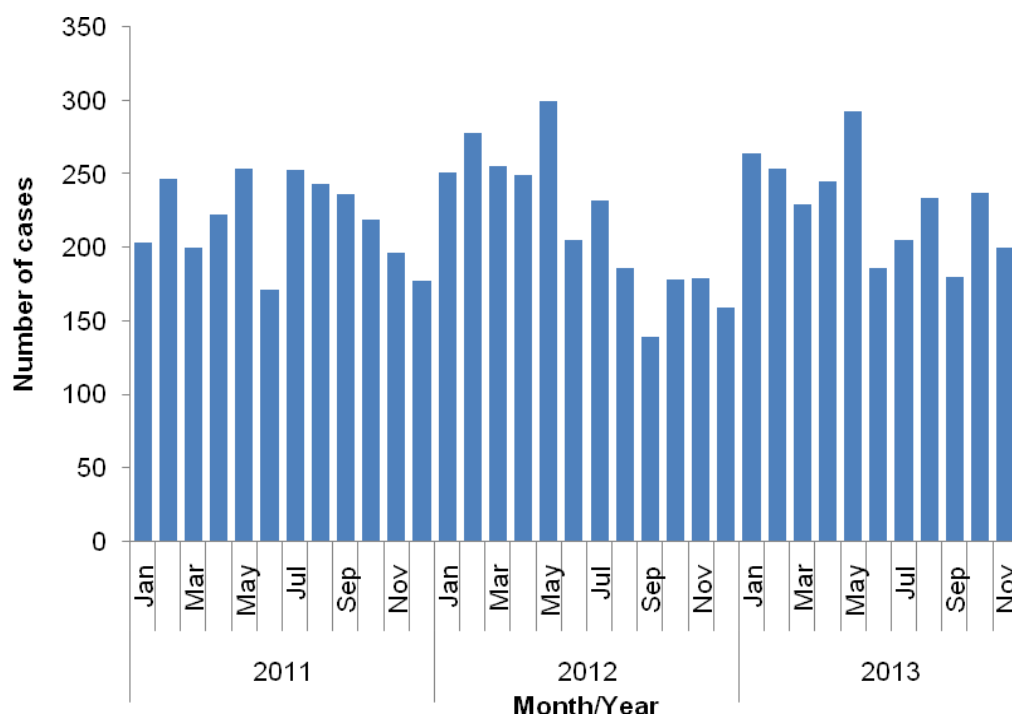


Figure 1: Frequency of laboratory-confirmed hepatitis A cases by month and year of specimen collection, public health sector, South Africa, 2011-2013.

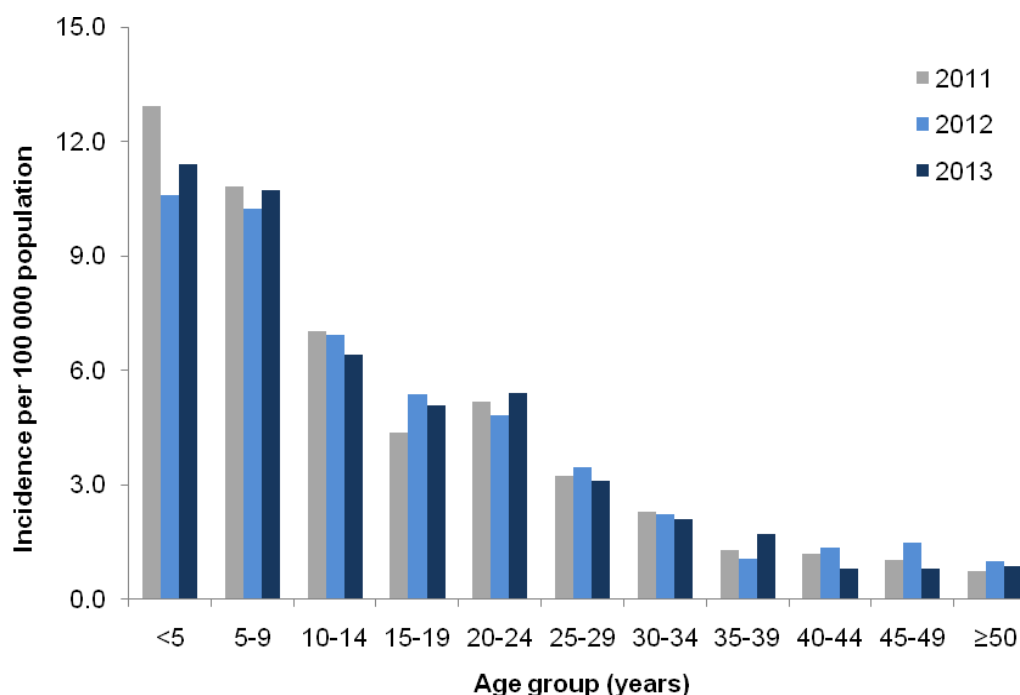


Figure 2: Age-specific incidence of laboratory-confirmed hepatitis A cases, public health sector, South Africa, 2011-2013.

Discussion

Analysis of laboratory-confirmed hepatitis A cases tested in the public health sector was restricted to laboratory-confirmed cases, and since laboratory testing for HAV is generally only performed for patients presenting with overt hepatitis or jaundice where testing is readily available, a minimum estimate of the total number of symptomatic infected persons and, by extension, a very small proportion of the actual number of hepatitis A infections across the country, is given.

The pattern of age distribution was consistent across the reporting period, with children <10 years of age constituting a higher proportion of cases, and children <5 years of age accounting for 25% of cases overall. Since only 5-20% of children <5 years of age develop symptoms following HAV infection that would prompt laboratory testing for hepatitis A, this suggests that HAV endemicity levels are still high in many communities with many persons being infected in childhood.

The number of laboratory-confirmed cases differed between provinces with the Gauteng and Western Cape provinces accounting for a higher proportion of cases.

This is likely multifactorial, including differential access to care between urban and rural areas, differences in protocols for laboratory testing and specimen collection, accessibility of laboratory testing facilities, and higher index of suspicion amongst certain groups of healthcare workers resulting in increased numbers of specimens submitted for testing. Of interest is that a decrease in incidence was noted in these two provinces over the reporting period, while an increase was noted in KwaZulu-Natal Province. This might be a consequence of incomplete data due to the migration of laboratory information systems (LIS) from DISA to TrakCare as well as the integration of historical LIS data from KwaZulu-Natal Province into the central NHLS LIS. It is possible that not all anti-HAV IgM-positive cases are reflected, resulting in lower case numbers during the LIS migration period.

Data presented here had several limitations. They relate only to laboratory-confirmed hepatitis A cases tested at NHLS laboratories, and do not include cases where no specimens were collected, or cases tested at private laboratories. It is likely that in many provinces the numbers of laboratory-confirmed hepatitis A cases are

disproportionately under-represented for several reasons, and therefore mask higher incidences of disease than estimated here. Information on the clinical presentation and/or severity of cases and mortality is rarely submitted to the laboratory and could therefore not be commented on.

Robust, representative hepatitis A serosurveys are well overdue. It is likely that heterogeneous pockets of susceptible and exposed persons co-exist in the South African context, given the broad range of socioeconomic conditions and associated safe water and sanitation availability. The routine inclusion of hepatitis A vaccine in the private practice childhood vaccine schedule (but not in the Department of Health Expanded Programme on Immunisation schedule) has additional implications, since childhood hepatitis A vaccination programmes have been consistently shown to decrease hepatitis A

incidence rates in all age groups of the population targeted owing to a marked herd immunity effect.^{1,4} It is expected that the incidence of hepatitis A in population groups of higher economic means that access private healthcare will experience an even lower incidence of infection than previously reported. As South Africa moves towards transitioning from an overall high endemicity to a lower level of transmission, hepatitis A outbreaks may become more frequent and widespread since shifts in the age-specific patterns of disease will result in an increasing proportion of susceptible adolescents and adults, often those living in urban areas with improved socioeconomic conditions.

Acknowledgements

The NHLS-CDW and NHLS laboratories are thanked for the provision of data.

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ANTIMICROBIAL RESISTANCE SURVEILLANCE FROM SENTINEL PUBLIC HOSPITALS, SOUTH AFRICA, 2013

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Introduction

Antimicrobial resistance (AMR) is a key public health concern that threatens effective treatment of antimicrobial infections, both locally and globally. Surveillance is conducted to determine the extent and pattern of resistance amongst the most important disease causing pathogens in humans.¹ The objectives of the AMR surveillance programme are to determine the number of cases reported from selected hospitals by month for selected pathogens and to describe antimicrobial susceptibility to the most important treatment regimens by pathogen by hospital.

Methods

All data were sourced from the National Health Labora-

tory Service (NHLS) Corporate Data Warehouse (CDW). This is a national repository for all public health hospitals in South Africa and contains archived data from two laboratory information systems (LIS), DISALAB and TrakCare.²

Bloodstream infections for the period January to December 2013 were extracted for the following pathogens: *Acinetobacter baumannii* complex, *Enterobacter cloacae* complex, *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Routine data were collected from sentinel sites (mostly academic sites) (table 1).

Table 1: Antimicrobial Resistance Surveillance participating hospitals by province, and their characteristics.

Hospital Site	Province	Academic Hospital	No of beds
Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)	Gauteng	Yes	1088
Chris Hani Baragwanath Hospital (CHBH)	Gauteng	Yes	3200
Dr George Mukhari Hospital (DGMH)	Gauteng	Yes	1200
Grey's Hospital (GH)	KwaZulu-Natal	Yes	530
Groote Schuur Hospital (GSH)	Western Cape	Yes	893
Helen Joseph Hospital (HJH)	Gauteng	Yes	700
Inkosi Albert Luthuli Central Hospital (IALCH)	KwaZulu-Natal	Yes	846
King Edward VIII Hospital (KEH)	KwaZulu-Natal	Yes	922
Mahatma Gandhi Hospital (MGH)*	KwaZulu-Natal	No	350
Nelson Mandela Academic Hospital/Mthatha Tertiary (NMAH)	Eastern Cape	Yes	520
RK Khan Hospital (RKKH)*	KwaZulu-Natal	No	543
Steve Biko Academic Hospital (SBAH)	Gauteng	Yes	832
Tygerberg Hospital (TH)	Western Cape	Yes	1310

* Non academic sites

Antimicrobial susceptibility test reporting was based on Clinical and Laboratory Standards Institute (CLSI) guidelines.³ The different laboratory methods used included Microscan, Vitek and disk diffusion. Owing to the two different LIS, each with its own coding system of organisms and antibiotics, as well as a lack of standardized data capturing across NHLS laboratories, extensive cleaning and recoding of data was necessary. Data cleaning involved creating unique patient identifiers, de-duplication and generation of patient-level data. Some data may be incomplete due to missing cases not captured on the LIS or non-standardized coding of pathogens and antibiotics.

Results

Data from antimicrobial susceptibility tests are summarised for: *Acinetobacter baumannii* complex (figure 1), *Pseudomonas aeruginosa* (figure 2), *Enterobacter cloacae* complex (figure 3), *Escherichia coli* (figure 4), *Klebsiella pneumoniae* (figure 5), *Staphylococcus aureus* (figure 6), *Enterococcus faecalis* (figure 7) and *Enterococcus faecium* (figure 8). For each organism, total number of cases by month, and susceptibility to selected antimicrobial agents with numbers and percentages (susceptible or resistant) per site were analyzed.

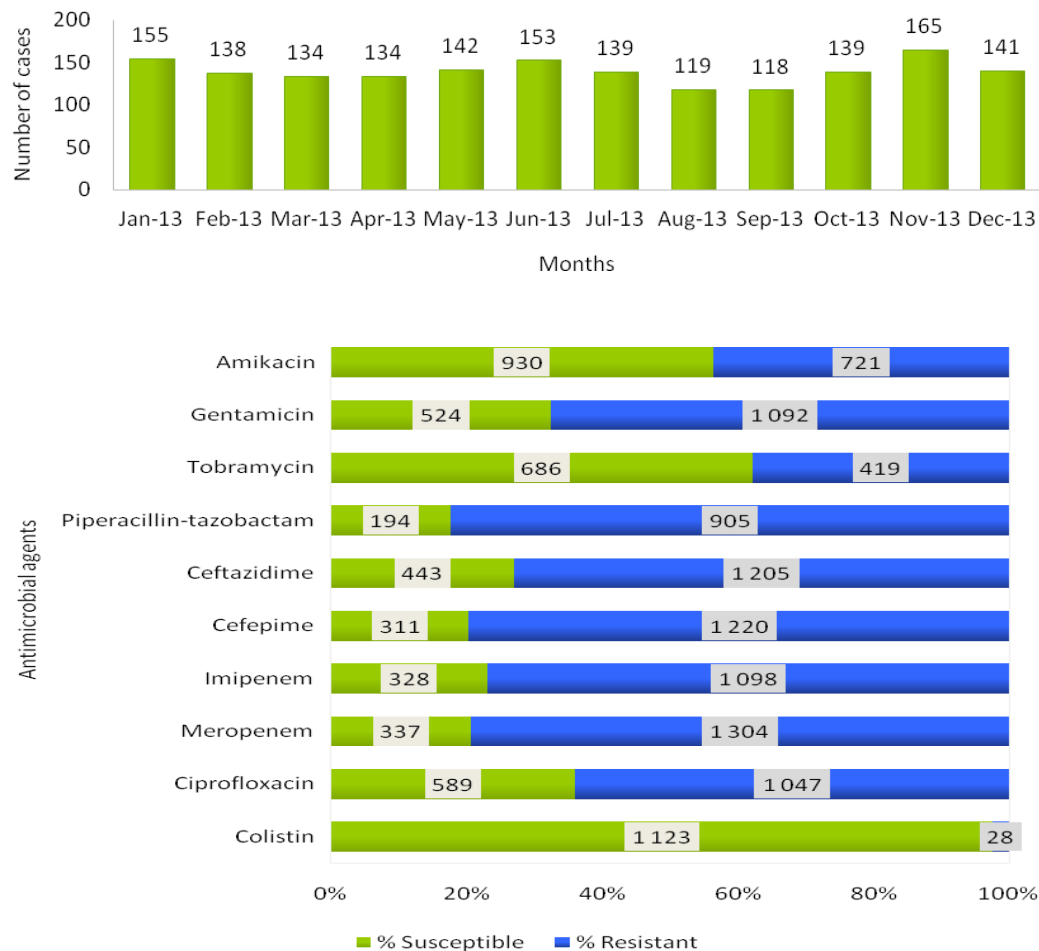


Figure 1: *Acinetobacter baumannii* cases by month, and numbers and percentages of susceptible and resistant *A. baumannii* complex isolates from blood cultures at public-sector sentinel sites, 2013. Total number of isolates analyzed = 1677.

Acinetobacter baumannii is resistant to the majority of antimicrobial agents listed owing to various mechanisms of resistance including: loss of outer membrane porins and permeability, efflux system, Amp C beta-lactamases and others. Resistance was high to imipenem, cefepime and ceftazidime, and a little lower to ciprofloxacin and amikacin. Colistin resistance was low for the period under review.

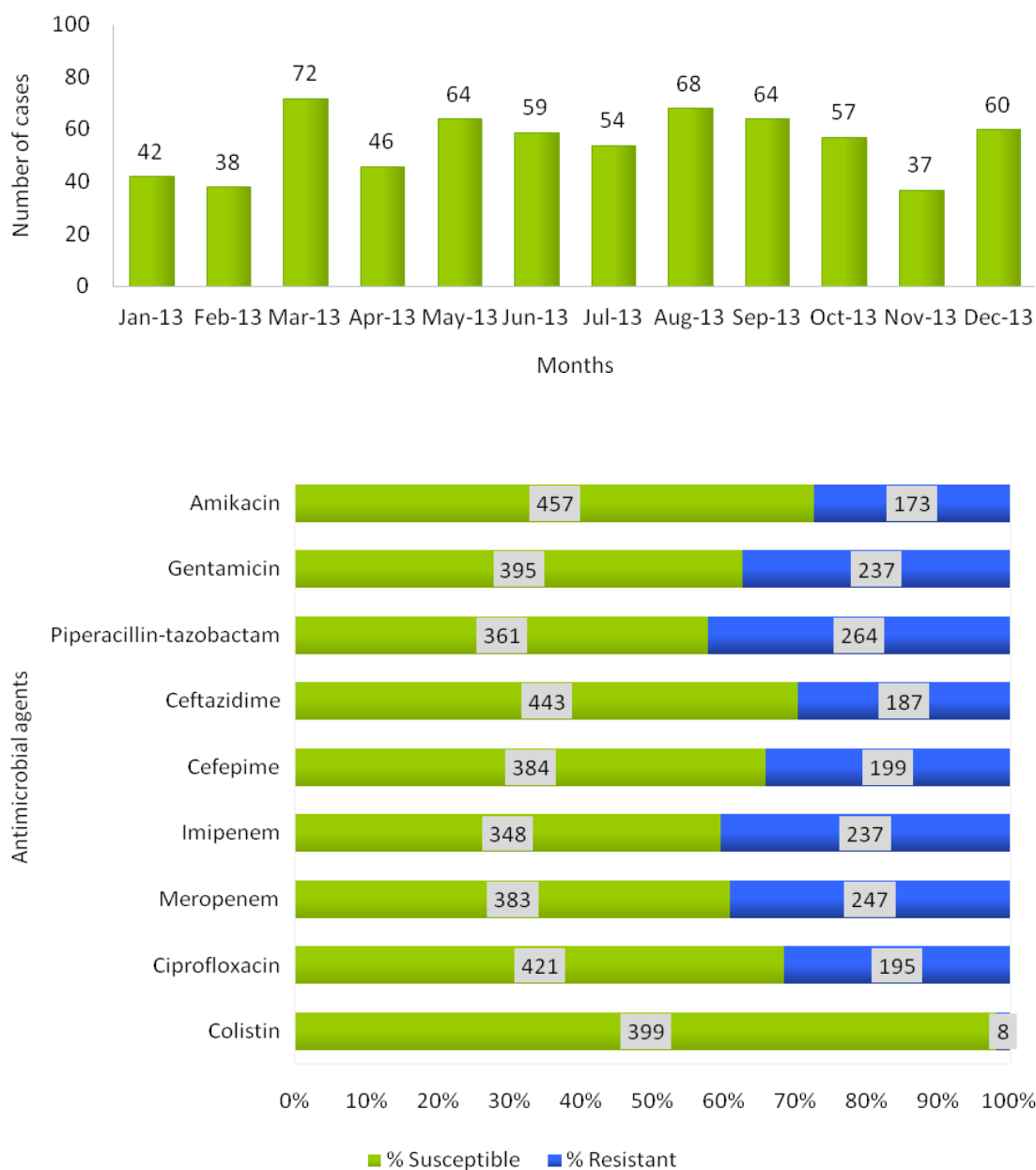


Figure 2: *Pseudomonas aeruginosa* cases by month, and numbers and percentages of susceptible and resistant *P. aeruginosa* isolates from blood cultures at public-sector sentinel sites, 2013. Total number of isolates analyzed = 661.

Pseudomonas aeruginosa isolates were moderately resistant to antimicrobial agents compared to *A. baumannii*. Resistances to ceftazidime, ciprofloxacin, piperacillin-tazobactam and imipenem were highest. Colistin resistance was lowest.

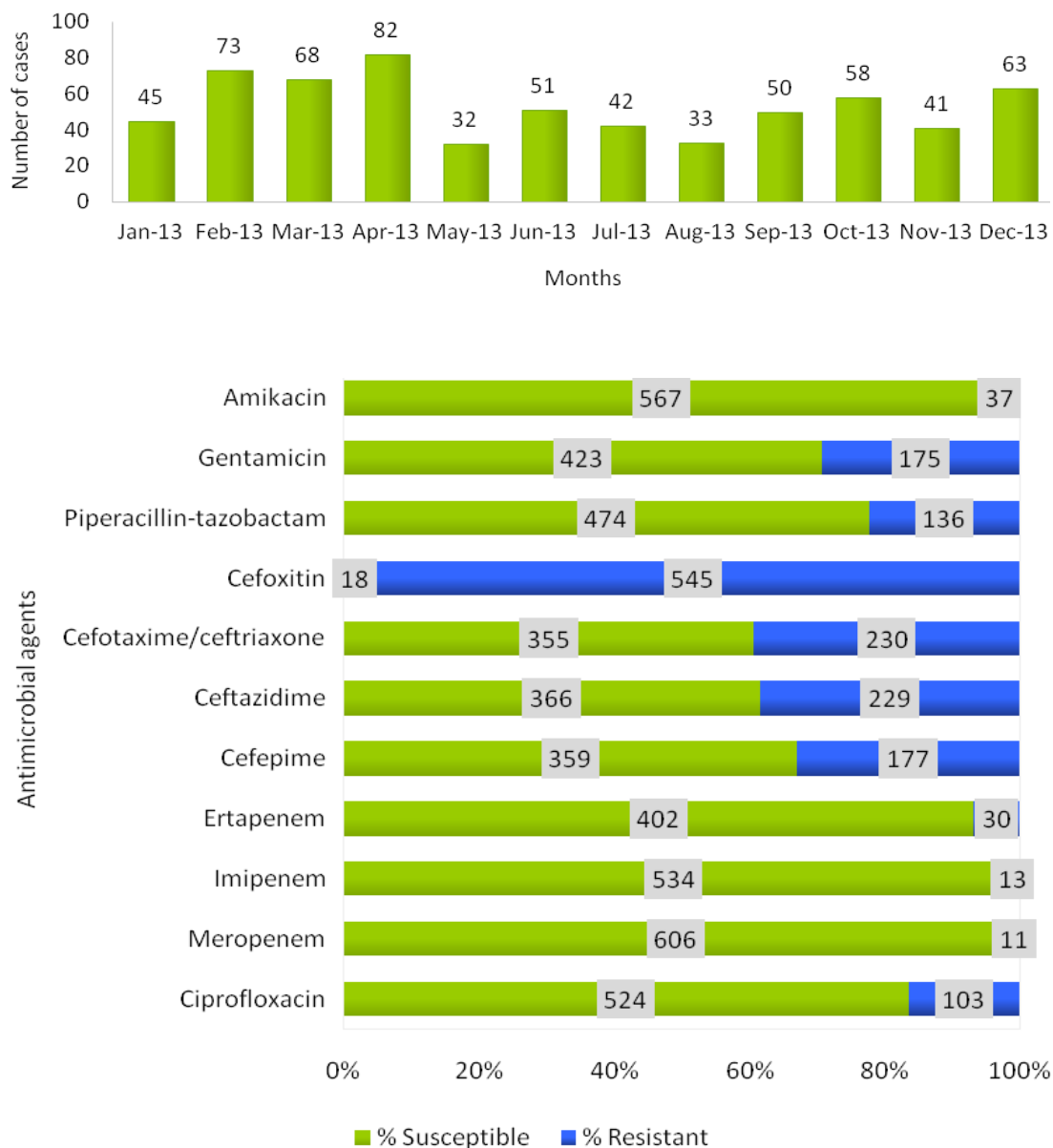


Figure 3: *Enterobacter cloacae* cases by month, and numbers and percentages of susceptible and resistant *E. cloacae* complex isolates from blood cultures at public-sector sentinel sites, 2013. Total number of isolates analyzed = 638.

The level of presumptive (no molecular confirmation) resistance of *E. cloacae* complex to ertapenem (7%) is of concern. Resistance to cefepime (33%) indicates the presence of de-repressed mutants resistant to all cephalosporins.

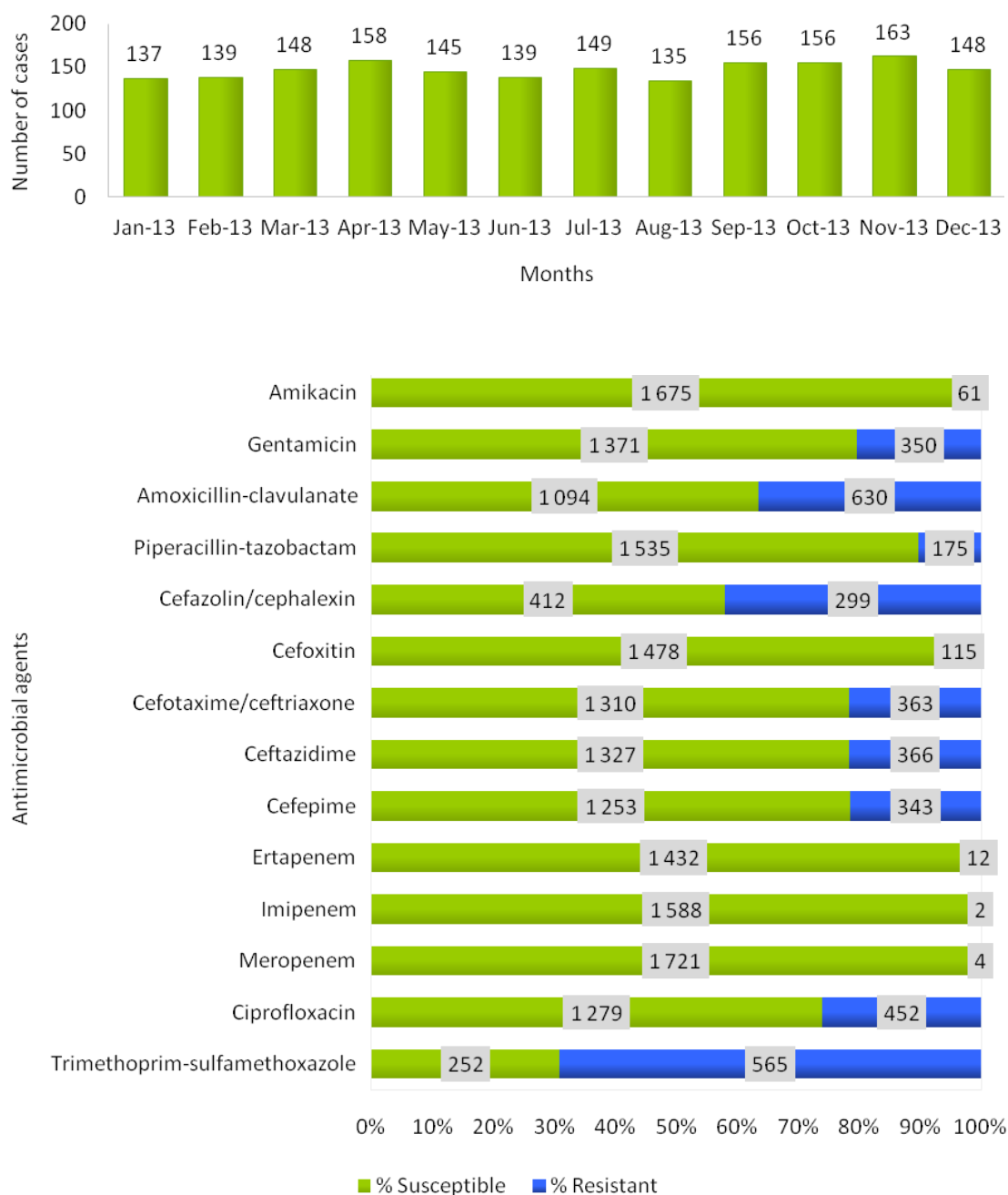


Figure 4: *Escherichia coli* cases by month, and numbers and percentages of susceptible and resistant *E. coli* isolates from blood cultures at public-sector sentinel sites, 2013. Total number of isolates analyzed = 1773.

Resistance to antimicrobials was high in *E. coli*. Resistance to amoxicillin-clavulanate as well as 1st and 3rd generation cephalosporins indicates the presence of extended spectrum beta-lactamases (ESBLs). Ciprofloxacin resistance is of concern.

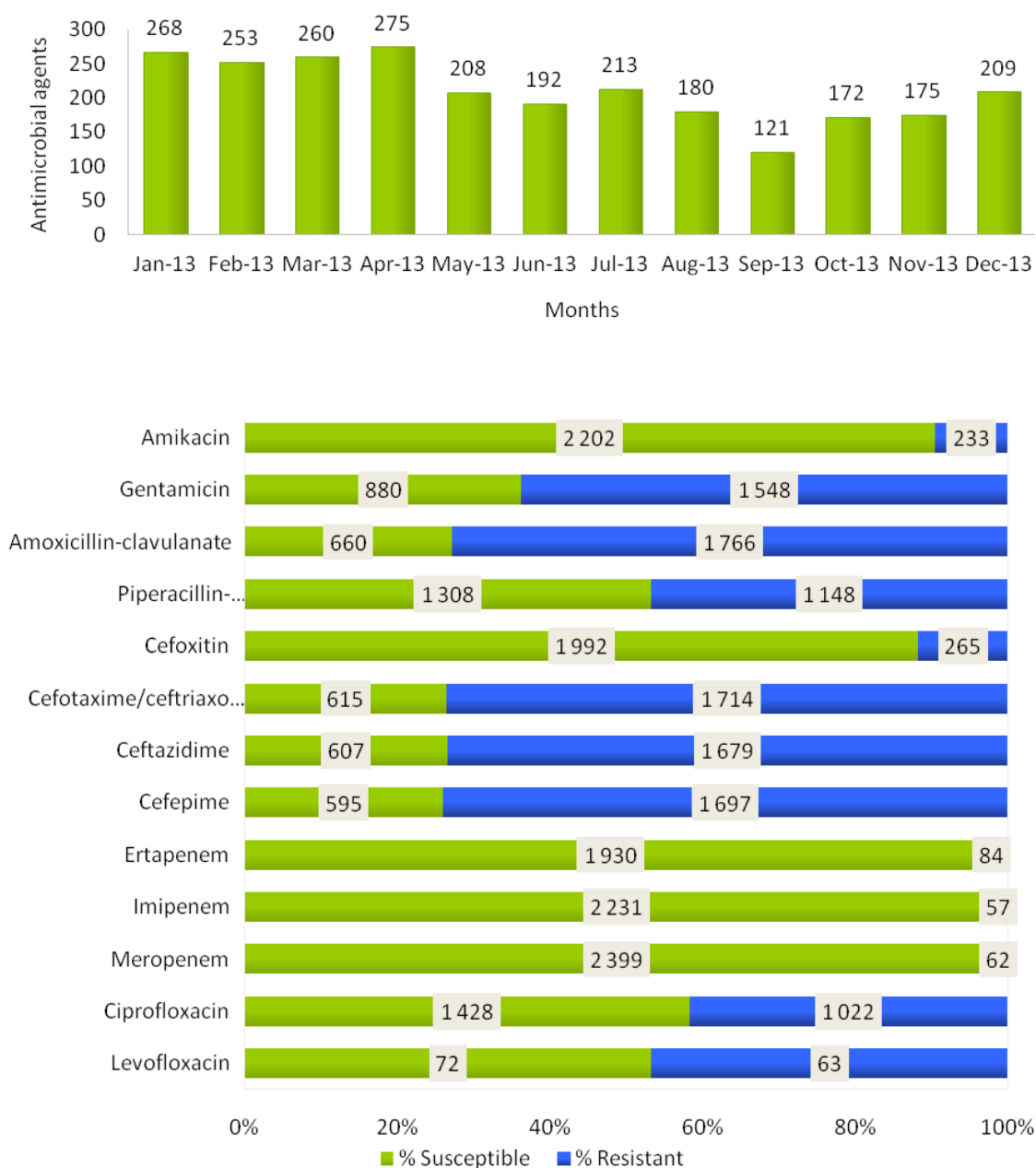


Figure 5: *Klebsiella pneumoniae* cases by month, and numbers and percentages of susceptible and resistant *P. aeruginosa* isolates from blood cultures at public-sector sentinel sites, 2013. Total number of isolates analyzed = 2526.

Klebsiella pneumoniae was resistant to multiple antimicrobials including ESBLs, ciprofloxacin and amikacin. Ertapenem resistance was low. Although resistance to other carbapenemases was very low, the rapid emergence of strains with carbapenemases production threatens the last line of therapeutic options. Thus, continuous monitoring of resistance needs to be implemented.

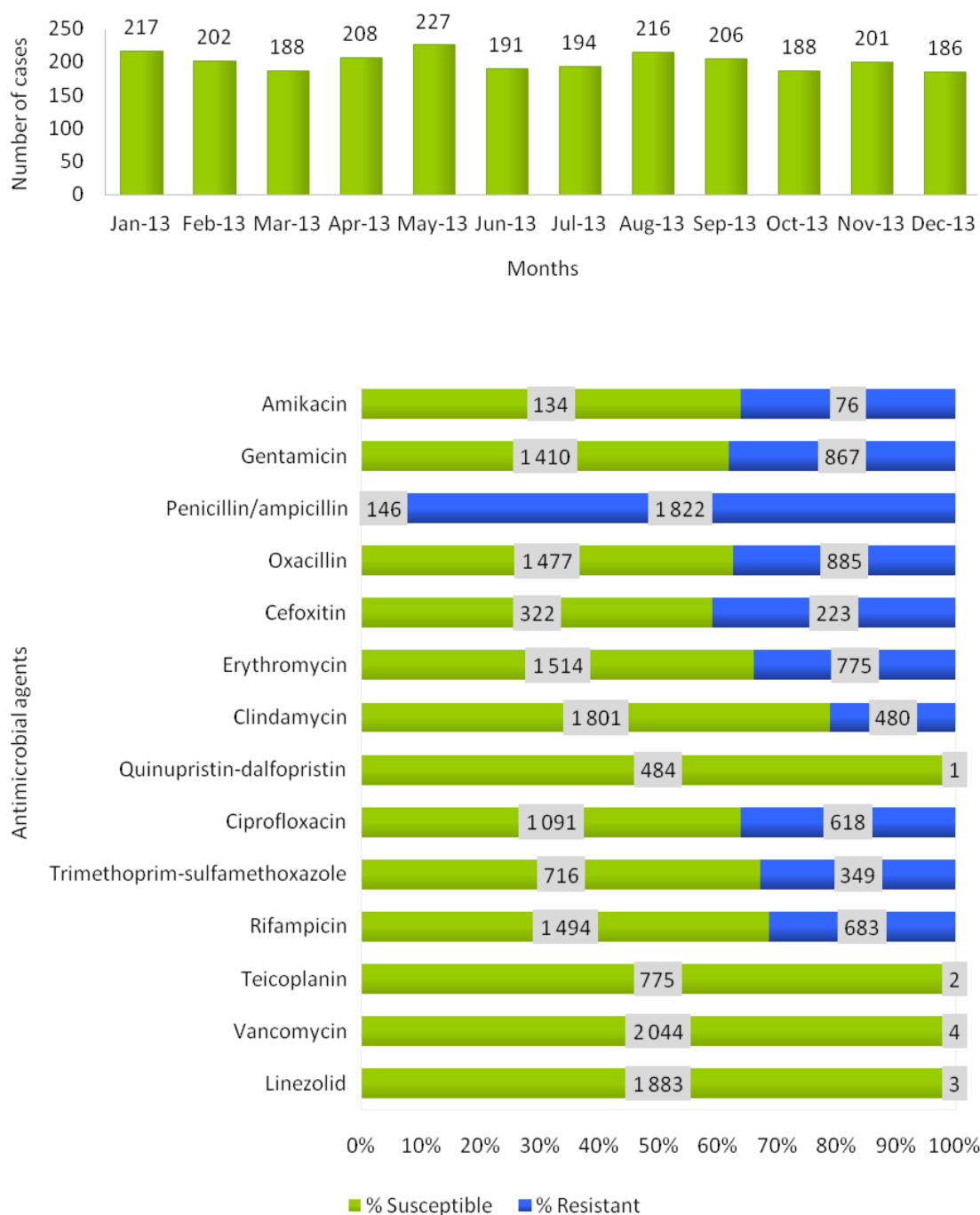


Figure 6: *Staphylococcus aureus* cases by month, and numbers and percentages of susceptible and resistant *S. aureus* isolates from blood cultures at public-sector sentinel sites, 2013. Total number of isolates analyzed = 2424.

Four *S. aureus* isolates were reported to be vancomycin resistant. However, this was not confirmed and data should be treated with caution. Resistances to methicillin/oxacillin and all other beta-lactams were recorded. Cefoxitin resistance was high indicating Methicillin-resistant *Staphylococcus aureus* (MRSA). Resistances to erythromycin and clindamycin were also recorded.

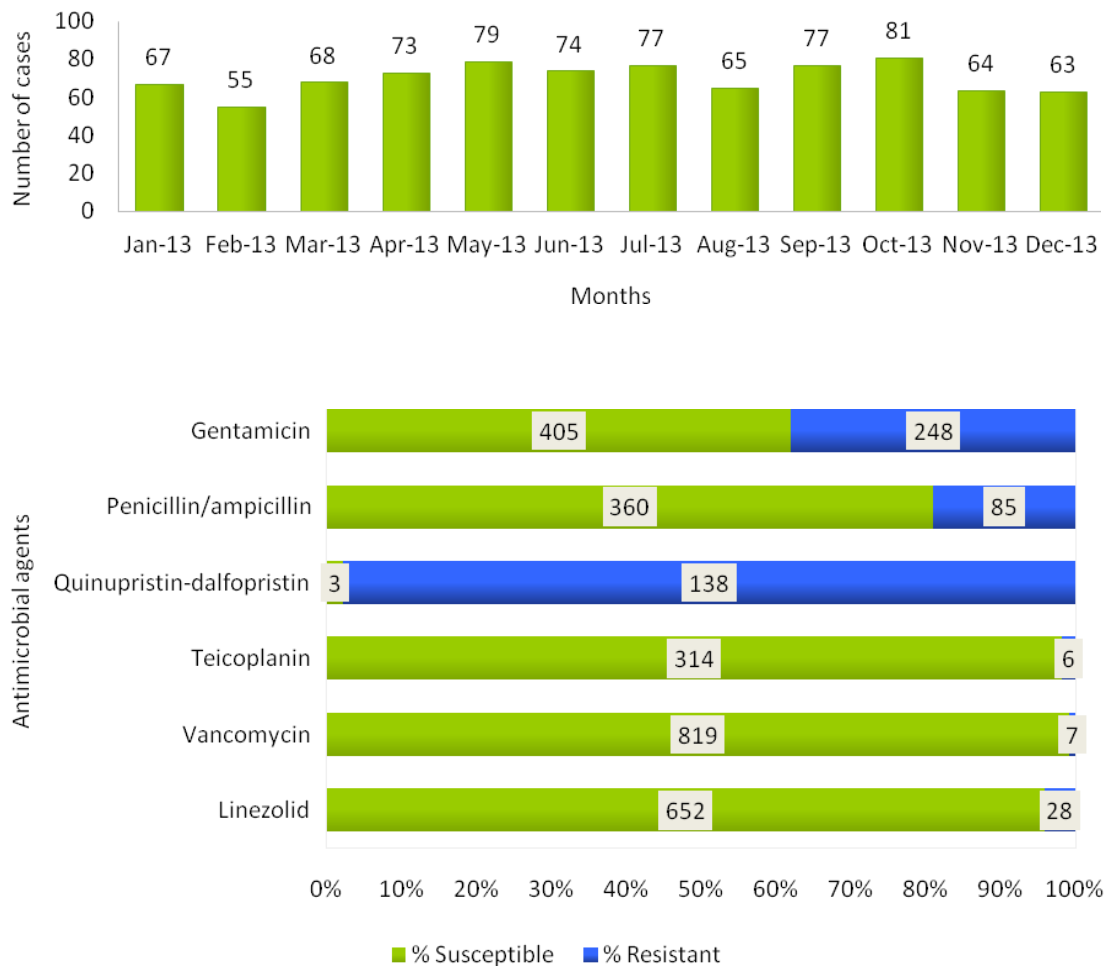


Figure 7: *Enterococcus faecalis* cases by month, and numbers and percentages of susceptible and resistant *E. faecalis* isolates from blood cultures at public-sector sentinel sites, 2013. Total number of isolates analyzed = 843.

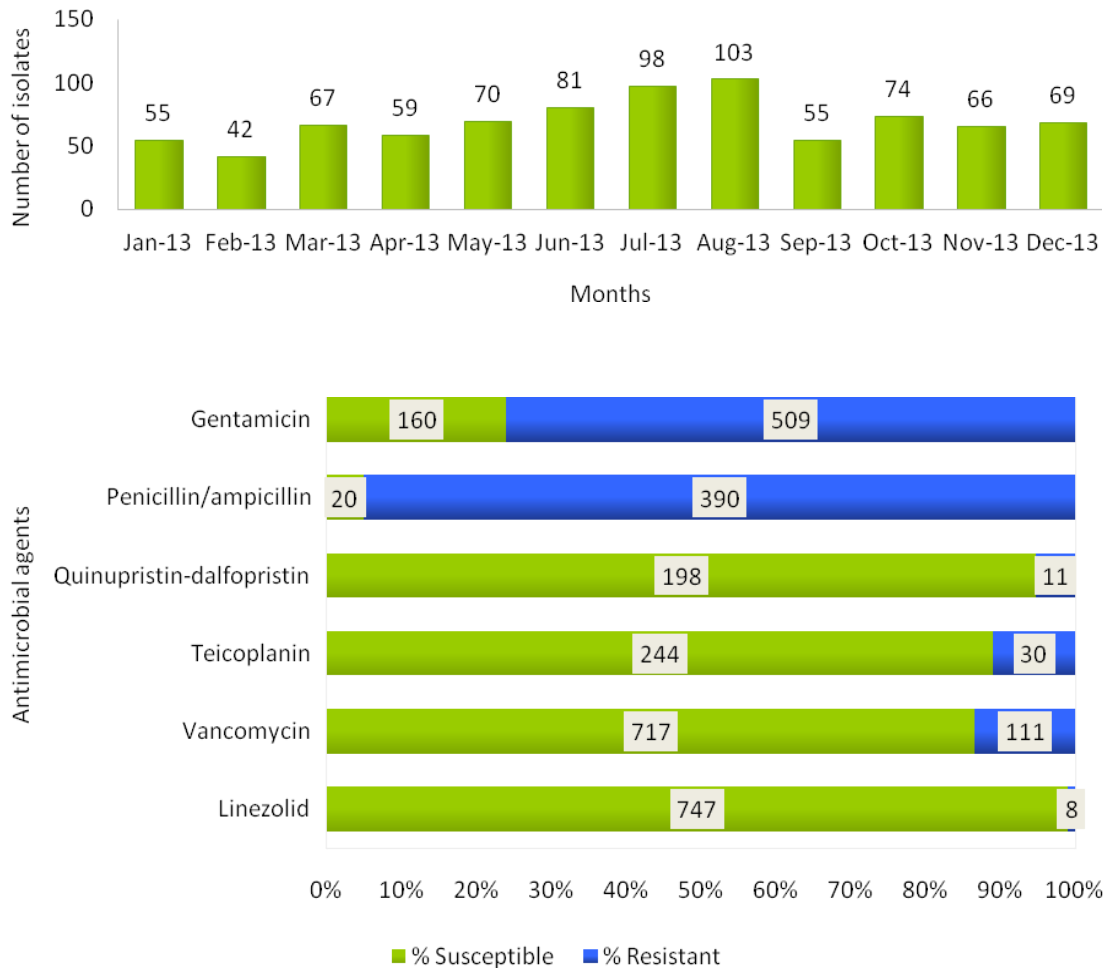


Figure 8: *Enterococcus faecium* cases by month, and numbers and percentages of susceptible and resistant *E. faecium* isolates from blood cultures at public-sector sentinel sites, 2013. Total number of isolates analyzed = 839.

Enterococci are intrinsically resistant to a broad range of antibiotics including cephalosporins, penicillins (*E. faecium*), sulfonamides, and low concentrations of aminoglycosides. Vancomycin resistant *E. faecium* was recorded which may indicate persistence of the strains or an outbreak.

Conclusion and final remarks

The data presented in this report highlighted the importance of surveillance for antimicrobial resistance patterns. Surveillance needs to be ongoing in order to identify trends as well as possible outbreaks.

Disclaimer

Data are reported as received through the CDW. No

clinical data or molecular data were available to distinguish between hospital-associated and community acquired infections.

Acknowledgements

The NHLS CDW team is acknowledged for cleaning the data and preparing the table and figures.

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ANOPHELES SPECIES COMPOSITION AND INSECTICIDE SUSCEPTIBILITY STATUS OF THE MAJOR MALARIA VECTOR ANOPHELES ARABIENSIS AT VLAKBULT, MPUMALANGA, 2012/13

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Introduction

Local malaria transmission in South Africa primarily occurs in three provinces: Limpopo, KwaZulu-Natal (KZN) and Mpumalanga.¹ Malaria in South Africa is categorized as seasonal, with peak transmission occurring between October and May, with the potential for outbreaks.¹

The primary vectors of malaria in South Africa are *Anopheles arabiensis* Patton (a member of the *An. gambiae* species complex) and *An. funestus* Giles (the nominal member of the *An. funestus* species sub-group).² These are controlled using insecticides based on the indoor residual spraying (IRS) technique.³ The development of insecticide resistance in target vector populations, primarily in northern KwaZulu-Natal^{4,5}, necessitates a resistance management strategy which includes ongoing malaria vector surveillance in all malaria affected provinces.³

Malaria vector surveillance includes the collection and identification to species of anopheline mosquitoes in affected regions. Collected specimens are then used for vector incrimination assessments and live specimens, or their progeny, are used for assessments of insecticide susceptibility. Data showing malaria vector species occurrence and insecticide susceptibility status can be used to plan effective malaria vector control strategies by province or district.

The aim of this study was to assess the insecticide susceptibility status of potential malaria vector species in Enhlanzeni district, Mpumalanga province.

Materials and Methods

Mosquito collections

Mosquitoes were collected, using standard collection methods, from Vlakbult (Nkomazi municipality that falls under Enhlanzeni district), Mpumalanga Province (25°38' 43.3" S, 31° 42' 00.2" E) one full week per month during November 2012, December 2012 and January 2013. During the period of the study, 640 cases were reported in Nkomazi municipality, 10 of which were reported from Vlakbult (Mpumalanga malaria control programme, unpublished data). Mosquitoes were identified to species using external morphology and standard molecular methods.⁶⁻⁸

Insecticide susceptibility tests

Adult anophelines obtained from wild-caught larvae of interest were assessed for insecticide susceptibility using the standard WHO procedure.⁹ Owing to small sample sizes, only two insecticides were used for assessments (4% DDT and 0.05% deltamethrin). These insecticides were in use by the Mpumalanga malaria control programme during the periods of collection.

Vector incrimination

Malaria vector incrimination is based on the detection of

Plasmodium spp. sporozoites in the salivary glands of wild-caught females previously identified to species. A standardized enzyme linked immunosorbant assay (ELISA)¹⁰ was used to detect the presence of *P. falciparum* circumsporozoite protein in wild female mosquitoes collected at the study site.

Results

A total of 130 mosquitoes was collected during the study period. Of these, 62 (47.6%) were morphologically identified as members of the *An. gambiae* complex, 26 (20%) were members of the *An. funestus* group, 3 (2.3%) were classified as 'other' anophelines and 39 (30%) were identified as culicine mosquitoes of no public health significance. Molecular PCR analysis revealed that 39 (62.9%) of the *An. gambiae* complex specimens were *An. arabiensis*, 8 (12.9%) were *An. quadriannulatus*, 4 (6.4%) were *An. merus* and 11 (17.7%) could not be identified to species (figure 1). The *An.*

funestus group specimens were primarily composed of *An. vaneedeni* (16/26 - 61.5%) followed by *An. rivolurum* (6/26 - 23.1%) and *An. leesoni* (4/26 - 15.4%). Specimens identified as *An. marshalli* and *An. demeilloni* contributed less than 5% to the total sample collected (table 1).

The bulk of the *An. arabiensis* samples (28/35 - 80%) were collected as larvae. These larvae were reared through to adults and exposed to either 4% DDT (n=22) or 0.05% deltamethrin (n=8). There were no survivors 24 hours post exposure to either insecticide suggesting full susceptibility.

A total of 32 adult *An. arabiensis* mosquitoes was tested for the presence of *P. falciparum* circumsporozoite protein using the ELISA technique. All of these mosquitoes tested negative.

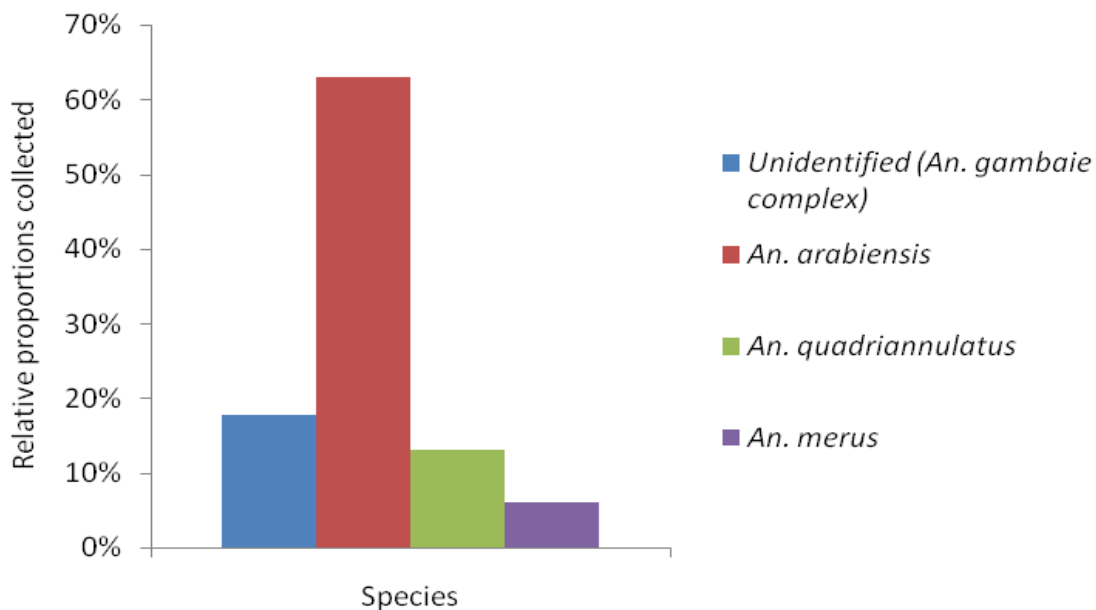


Figure 1: Relative proportions of members of the *Anopheles gambiae* complex collected at Vlakbult, Mpumalanga Province, during three collection periods from November 2012 to January 2013 (n=62). 18% could not be identified by PCR but were morphologically identified as *An. gambiae* complex.

Table 1: Numbers of mosquitoes by species collected from Vlakkbult, Mpumalanga Province, South Africa, during three collection periods from November 2012 to January 2013.

Species	Number collected	Percentage (%)
Unidentified to species level	11	8.5%
<i>An. arabiensis</i>	39	30%
<i>An. quadriannulatus</i>	8	6.2%
<i>An. merus</i>	4	3.1%
<i>An. vaneedeni</i>	16	12.3%
<i>An. lesoni</i>	4	3.1%
<i>An. rivulorum</i>	6	5.4%
<i>An. demeilloni</i>	2	2.3 %
<i>An. marshalli</i>	1	
Culex sp.	39	30%
Total	130	100%

Discussion

Vlakkbult is generally considered to be a low risk malaria zone that is sprayed annually using IRS. Nevertheless, the major malaria vector *An. arabiensis* was collected there albeit in low numbers.

It is important to note that the majority of the *An. arabiensis* sampled were collected as larvae. This may be a consequence of unfavorable weather conditions affecting the sampling of adult mosquitoes. The *An. arabiensis* sampled showed full susceptibility to DDT and deltamethrin based on standard WHO criteria.⁹ However, the sample sizes obtained were too small to make definitive statements concerning the insecticide susceptibility status of *An. arabiensis* at Vlakkbult, and further monitoring of insecticide susceptibility will be needed. WHO recommends that a minimum sample size of 100 mosquitoes per species per insecticide (excluding controls) be assayed in order to draw firm conclusions on the presence or absence of insecticide resistance in target populations.⁹

Plasmodium falciparum circumsporozoite analysis revealed that none of the adult female *An. arabiensis*

collected during this study were *P. falciparum* infective. This finding should however be interpreted with caution. The sample size for this study was small and if a low entomological infection characterizes this population, then a small sample size is not likely to yield any sporozoite positive females.

It is concluded that locally acquired malaria at Vlakkbult, Mpumalanga Province, is most likely transmitted by *An. arabiensis* and that there was no evidence of resistance to deltamethrin or DDT in the local *An. arabiensis* population during the sampling period. These data, however, should be treated with caution due to small sample sizes.

Acknowledgements

The following institutions and individuals are acknowledged for their contributions to this study: South African Field Epidemiology and Laboratory Training Programme; Vector Control Reference Laboratory of the National Institute for Communicable Diseases; University of Pretoria; National Department of Health; Mpumalanga Malaria Control team.

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Table 1: Provisional number of laboratory confirmed cases of diseases under surveillance reported to the NICD - South Africa, corresponding periods 1 January - 31 March 2013/2014*

Disease/Organism	1 Jan to 31 Mar, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa	
Anthrax	2013	0	0	0	0	0	0	0	0	0	0	
	2014	0	0	0	0	0	0	0	0	0	0	
Botulism	2013	0	0	0	0	0	0	0	0	0	0	
	2014	0	0	0	0	0	0	0	0	0	0	
<i>Cryptococcus spp.</i>	2013	179	60	521	467	28	95	9	68	140	1567	
	2014	170	54	418	411	28	83	12	55	220	1451	
<i>Haemophilus influenzae</i> , invasive disease, all serotypes	2013	6	2	23	16	0	2	1	0	23	73	
	2014	6	4	24	18	0	4	2	1	20	79	
<i>Haemophilus influenzae</i> , invasive disease, < 5 years	Serotype b	2013	1	0	2	2	0	0	0	0	2	7
		2014	1	1	2	2	0	0	1	0	4	11
Serotypes a,c,d,e,f	2013	0	0	1	0	0	0	0	0	1	2	
	2014	0	0	1	1	0	0	0	0	0	2	
Non-typeable (unencapsulated)	2013	0	0	3	0	0	0	0	0	3	6	
	2014	1	0	10	3	0	1	0	0	4	19	
No isolate available for serotyping	2013	1	0	6	2	0	2	1	0	2	14	
	2014	4	3	11	12	0	3	1	1	12	47	
Measles	2013	0	0	0	0	0	0	0	0	0	0	
	2014	0	0	0	1	0	0	0	0	1	2	
<i>Neisseria meningitidis</i> , invasive disease	2013	3	2	6	7	0	1	1	0	8	28	
	2014	11	2	7	1	0	0	0	0	9	30	
Novel Influenza A virus infections	2013	0	0	0	0	0	0	0	0	0	0	
	2014	0	0	0	0	0	0	0	0	0	0	
Plague	2013	0	0	0	0	0	0	0	0	0	0	
	2014	0	0	0	0	0	0	0	0	0	0	
Rabies	2013	0	2	0	1	1	1	0	0	0	5	
	2014	1	0	0	0	1	0	0	1	0	3	
<i>Salmonella typhi</i> **	2013	0	1	10	5	0	2	0	0	5	23	
	2014	0	0	16	4	0	5	0	0	6	31	
<i>Streptococcus pneumoniae</i> , invasive disease, all ages	2013	67	33	158	83	4	19	9	19	91	483	
	2014	50	34	178	86	2	22	5	20	104	501	
<i>Streptococcus pneumoniae</i> , invasive disease, < 5 years	2013	16	9	43	13	1	2	2	8	11	105	
	2014	6	6	43	24	1	4	2	5	16	107	
<i>Vibrio cholerae</i> O1	2013	0	0	0	0	1	0	0	0	0	1	
	2014	0	0	0	0	0	0	0	0	0	0	
Viral Haemorrhagic Fever (VHF) Crimean Congo Haemorrhagic Fever (CCHF)	2013	0	2	0	0	0	0	0	1	0	3	
	2014	0	1	0	0	0	0	0	0	0	1	
Other VHF (not CCHF)	2013	0	0	0	0	0	0	0	0	0	0	
	2014	0	0	0	0	0	0	0	0	0	0	

Footnotes

*Numbers are for cases of all ages unless otherwise specified. Data presented are provisional cases reported to date and are updated from figures reported in previous bulletins.

Provinces of South Africa: EC – Eastern Cape, FS – Free State, GA – Gauteng, KZ – KwaZulu-Natal, LP – Limpopo, MP – Mpumalanga, NC – Northern Cape, NW – North West, WC – Western Cape

0 = no cases reported

**Laboratory-based surveillance for *Shigella* and *Salmonella* spp other than typhi has been discontinued as of 2014

Table 2: Provisional laboratory indicators for NHLS and NICD, South Africa, corresponding periods 1 January - 31 March 2013/2014*

Programme and Indicator	1 Jan to 31 Mar, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Acute Flaccid Paralysis Surveillance											
Cases < 15 years of age from whom specimens received	2013	14	5	16	18	5	9	4	1	6	78
	2014	16	10	17	22	5	8	6	4	5	93

Footnotes

*Numbers are for all ages unless otherwise specified. Data presented are provisional numbers reported to date and are updated from figures reported in previous bulletins.

Provinces of South Africa: EC – Eastern Cape, FS – Free State, GA – Gauteng, KZ – KwaZulu-Natal, LP – Limpopo, MP – Mpumalanga, NC – Northern Cape, NW – North West, WC – Western Cape

Monitoring for the presence of polio in a country is based on AFP (acute flaccid paralysis) surveillance – the hallmark clinical expression of paralytic poliomyelitis. The clinical case definition of AFP is an acute onset of flaccid paralysis or paresis in any child under 15 years of age. AFP is a statutory notifiable disease and requires that 2 adequate stool specimens are taken as soon as possible, 24 to 48 hours apart, but within 14 days after onset of paralysis, for isolation and characterisation of polio virus. The differential diagnosis of AFP is wide, the most common cause of which is Guillain-Barre Syndrome. The incidence of AFP in a population has been studied in a number of developing countries and WHO have determined, as a result of these studies, that the criterion for adequate surveillance of AFP is 2 cases per 100 000 population of children less than 15 years of age (it was formerly 1 per 100,000 but this was thought to be inadequately sensitive).

The Communicable Diseases Surveillance Bulletin is published by the National Institute for Communicable Diseases (NICD) of the National Health Laboratory Services (NHLS), Private Bag X4, Sandringham, 2131, Johannesburg, South Africa.

Suggested citation: [Authors' names or National Institute for Communicable Diseases (if no author)]. [Article title]. Communicable Diseases Surveillance Bulletin 2014; 12(2): [page numbers].

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